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THE ANALYSIS OF THE RELATIVE GROWTH GRADIENTS AND CHANGING FORM OF GROWING ORGANISMS: ILLUSTRATED BY THE TOBACCO LEAF*

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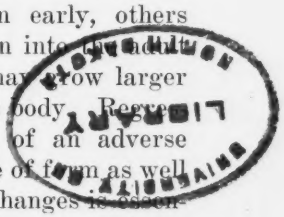
THE size and shape of plants and animals distinguish them from each other, and from these differences derives much of classification, comparative biology and even the recognition of individuals. Some organisms attain the proportions of the adult configuration early, others change slowly from the infantile pattern into the adult form. Claws, antlers, or other parts may grow larger and out of proportion to the rest of the body. Regression in form often denotes the effect of an adverse environment and senility involves change of form as well as function. The analysis of the form changes is essential to an understanding of the growth of organisms. This paper presents a method for this analysis which relates the transformed coordinate method with the allometric expression of growth gradients.



I

Toward the end of the nineteenth century it was discovered that a parabolic curve could represent the growth of organisms and of their component parts. About ten years ago Huxley, Teissier and Needham revived interest in this curve for the analysis of growth. They empha-

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sized the unequal growth resulting from gradients; calling it at first heterogonic growth, later allometry (Huxley, Needham and Lerner, 1941). The constant k, of the equation $y = bx^k$, is the ratio of the specific[1] growth rate (dy/ydt) of the part (y) with respect to that (dx/xdt) of the whole organism (x). Regions and individuals have been compared and the index has been used as an aid in unraveling evolutionary history and for the classification of animals. A critical and systematic evaluation of the use of this equation has been made by Kavanagh and Richards (1942).

It was soon observed that if values of the relative growth rate k were obtained for a series of parts arranged in order along the organism, the values usually changed systematically from one end of the series to the other. The rate of change in one series might be different from that in another series at right angles to the first. Centers at which k was a maximum were found. These studies were made by comparing fairly large portions of the organism and proceeding as though the value of k were constant within each such portion. It is reasonable to expect that the value of k will be found to vary continuously from point to point within such portions, and that a more accurate knowledge of the growth will be obtained if the analysis is extended to take account of this continuous variation.

Thompson (1915, 1917) demonstrated that an outline of a part of an organism on a coordinate grid could be deformed or transformed by mathematical methods into the outlines of different but related species. De Conick (1936) used transformation to assist in defining the limits for variation separating one taxonomic species from another. A similar method was used by Richards and Riley (1937) to recover the coordinates of developing forms for comparison with the adult form. Such transformations emphasize the development of form and

[1] The term "relative growth" has been used for the growth rate per unit of quantity (dy/ydt) and for the ratio of such growth of a part with respect to that of the whole organism. To prevent ambiguity we will call the former (change in growth/unit of growth/unit of time) the *specific* growth rate.

are helpful in drawing attention to the relative growth activity of different regions.

It is obvious that there must be a connection between these two approaches and that knowledge of the relations is essential to the analysis of the growth of organisms.

II

Suppose tiny bits of material to be embedded in the growing substance, particles so small as not to interfere with the normal growth process, but which do not themselves grow. By choosing coordinate axes (which are considered unchanged throughout the growth) for reference, we can analyze the changes in the geometrical configuration formed by the positions of these markers as growth proceeds. Such experiments have been accomplished by marking rectangular networks on young leaves and by noting the shape and size changes of vitally stained tissues.

Gradients in the relative growth rate imply corresponding gradients in the specific growth rate. Specific[1] rates simplify the analysis and will be used hereafter. The mutually perpendicular axes will be called x, y, and z. The position of a point at a given time can be expressed by these coordinates and time (t). Such a point as P_1 in Fig. 1 will move a small distance in some direction in a short interval of time. Nearby points will also be moving from growth, but in general with slightly different speeds and in slightly different directions. As a result the distance between two such points as P_1 and P_2 will be continually changing. The absolute rate of increase of length of this segment is $d\Delta s/dt$; the specific growth rate is $d\Delta s/\Delta s dt$. When Δs becomes infinitesimally small, the specific growth rate approaches a limit which may properly be called the elemental specific growth rate. This rate is given by a formula derived in the appendix. There it is shown that the value of the rate is in general different in different directions from P_1, so that a tiny sphere centered at P_1 and growing for

a short time will not remain spherical. It is possible to determine the directions of maximum and minimum specific rates and it turns out that these are always at *right angles to each other.*

For the special case in which the rate is independent of the direction the term *isotropic* may be used. Under isotropic growth a small sphere centered at P and growing for a short time would remain spherical. Under *non-*

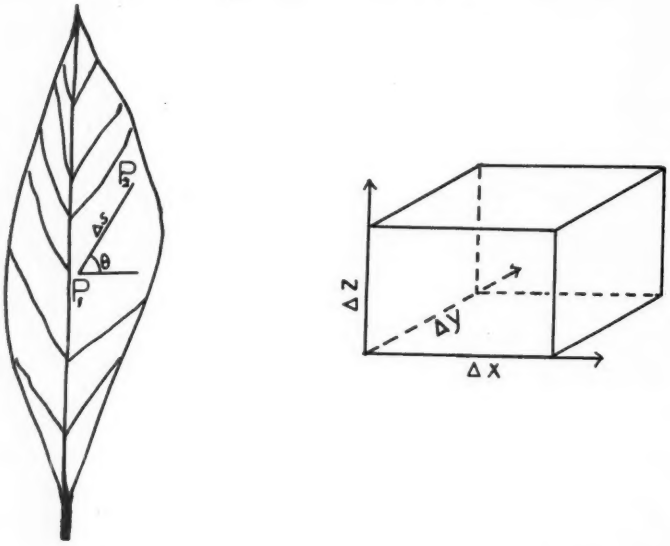

FIG. 1. Illustration of the directions of growth defined in the text.

isotropic growth the sphere would be deformed into some non-spherical shape.

Isotropic growth should not be mistaken for what is sometimes called *isogonic* growth, or change in size but not in shape. Although in isotropic growth a small sphere remains spherical for a short period of growth, neighboring spheres may not grow at the same rate, so that the gross form of the organism is not necessarily maintained. Isogonic growth is a special case of isotropic growth in which the specific growth rate in length

is the *same at every point* throughout the organism, as well as being the *same in any direction* from any given point. The specific growth rate in volume and the specific growth rate in area of a surface can also be expressed in terms of the motions of the points.

III

Few studies of growth are sufficiently complete to provide the basic measurements for a complete growth analysis. Our method is more clearly demonstrated by the analysis of growth restricted to two dimensions than if all three are involved. The best material available to illustrate the method was that on the tobacco leaf of Avery (1933) and we are grateful to Professor Avery for permission to use his material. A young tobacco leaf was marked with a rectangular network on its upper surface. As it grew to maturity the network was deformed by the growth process and Avery's figures 28–31a give four growth stages which will be used to illustrate the analytical method.

The data are from one leaf and we were unable to secure the original measurements, but were forced to rely on enlargements of Avery's published drawings. While recognizing the limitations of the data, we have prepared the present discussion with the idea of illustrating the method and have not hesitated to point to suggested conclusions whose complete verification would require more extensive and more nearly accurate data than were available.

Although growth in the neighborhood of the center of the leaf is continuous, the nature of the markings did not make it possible to use this fact in the analysis. Therefore each half of the leaf was analyzed in turn and the midrib data were used with each half. Slight apparent discontinuities resulting from this fact will be seen.

A description of the procedure used in applying the general formulae to this example is given in the appendix.

The rectangles, marked on the leaf surface, changed shape as the leaf increased in size and attained the adult

form. The corners of the rectangles are the reference points used in the analysis. For convenience the lines originally at right angles to the midrib have been numbered from 0 to 12 starting at the base of the leaf.

The distribution of values of the specific growth rate in area is shown in each of the four stages of Fig. 2 by solid lines along which the rate is constant, in a manner similar to the use of isotherms on a weather map to indicate places of equal temperature. The curves marked "100" are those of approximately the maximum rate at

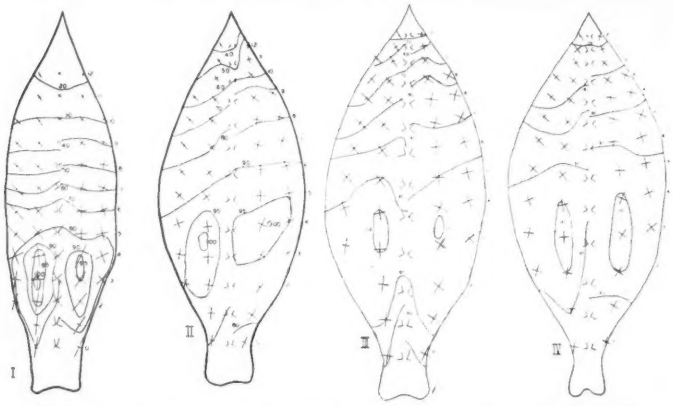

FIG. 2. Growth of the tobacco leaf; leaves drawn to the same length.

each stage; the numbers "95," "90," etc., indicate that the rate on the corresponding curve is 95 per cent., 90 per cent., etc., of that of the curve marked "100." As a result of the separate analysis of the two halves of the leaf, the curve for a given value is sometimes broken at the midrib; the two parts of each curve have then been joined by a dotted line.

The tip of the leaf at the first stage, figure 2, is growing at less than 20 per cent. of the maximum area rate, while at the fourth stage the tip is growing at a little under 70 per cent. of the maximum area rate. The position of maximum rate of area growth remains the same near the lateral marked 3 for all stages. A larger region for

maximum area rate occurred on the left side at stage II and resulted in asymmetry of the leaf as the smaller compensatory growth at stage III in this region did not remove the distortion. As the full size is attained, stage IV, the regions of maximum rate are nearly equal.

The contours illustrate clearly how small differences or gradients in the rate of growth can distort the characteristic symmetry, and the regulatory changes that restore the symmetry of the leaf. For convenience the

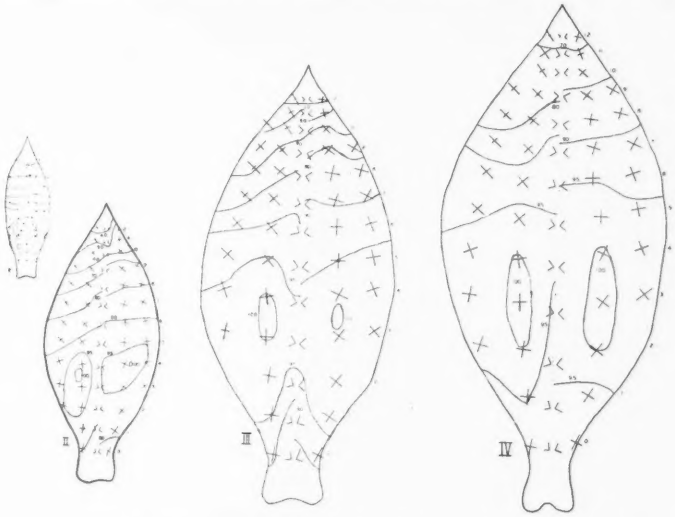

FIG. 3. Growth of the tobacco leaf; size of leaves proportional to their length at each stage of growth.

stages were made all the same size in Fig. 2. The true relative size of the leaves is shown in Fig. 3 and the importance of the changes in growth is more readily understood even though it is difficult to read the numerical data.

The nature of the specific growth rate in length is shown at each point by a pair of crossed lines. The length of each line is proportional to the specific growth rate in length in that direction. The directions are respectively those of maximum and of minimum rate at

each point and are at right angles to each other. Due to the separate analysis of the two halves of the leaf, two sets of values were obtained for each point at the midrib; these are shown by half-crosses slightly separated. The specific growth rates for the several points may be compared for the four stages with respect to the relative magnitude and the direction of growth. Note that the maximum rates for the outer points at 2 and 3, especially at stage III, are toward the midrib. This is as it should be; the vascular bundles likewise turn in the same direction, as shown clearly by figure 41 of Avery's paper (1933). The intensity of growth of the leaf axis is depicted graphically; likewise the decreasing gradient toward the tip of the leaf. A striking characteristic of the growth is that the strongly non-isotropic growth of the early stages tends toward isotropism as the leaf grows older. Other interesting and useful comparisons are possible. The active growth regions shown in our figures correspond with the changes in the underlying tissues revealed by the histological studies of Avery (1933).

The ratio of the relative growth rate of a part (growth per unit of growth) to that of the whole would give the quantity (k) of the allometric equation for the relative growth of that particular region to the whole organism. Little would be gained from computing these because different numerical values would be obtained at different stages and from those regions within a stage not growing at the same rate. The k's were computed by Avery for segment area to total leaf area and for length to width of segment; comparing the four stages by pairs. The tabulated values do not reveal the uniformity of the growth as readily as our figures. The more general analysis provides the numerical data to obtain the k's of the allometric method, when these are desired, with the advantage of information regarding the constancy of k (*cf.* Richards, 1935).

Connecting the several points would give the network and would reveal the transformation from stage to stage

due to the growth of the leaf and mathematical expressions for the transformation could be derived should they be required. This aspect is the method of Thompson (1917).

Thus the analytical method derived in the appendix and illustrated above includes both the transformed coordinate and the allometric methods. Other comparisons of the growth are possible. The method may be used for animal growth as well as that of plants and for all three dimensions when adequate measurements of a growing organism become available.

IV

The method becomes somewhat simpler in special cases, such as the growth rotationally symmetrical to its axis. This case has been investigated mathematically, although we have found no suitable data for testing. Certain instructive results may be obtained without the use of data or the presentation of the derivation. Sinnott (1936) concluded for the growth of the pine that, "Evidently the linear relation of pith to the whole could not persist much further without causing a reduction in the absolute size of the tissues outside of the pith." His conclusion was based on measurements of diameters rather than areas. While it is qualitatively true for areas as well, quantitatively the decrease in *area* of the outer bands of tissue will begin much later than the decrease in width. Changes in area rather than in radius may be a better indication of growth.[2] The question of isotropy of symmetrical growth of the type considered in this section shows that a condition for *isotropic growth* is that the growth in any plane section perpendicular to the axis is *isogonic*.

Geometrical change alone, as stated before, may not give a completely satisfactory picture of the underlying

[2] Strictly speaking, Sinnott's study is one of relative size rather than of relative growth, since the data are of Type B of Kavanagh and Richards (1942) classification. Since his successive individuals may be considered to form a series, the same principles hold as in the case of actual relative growth.

growth activity. The change in size at a given point is due to both the functional activity of the cells located there and to the forces of stretch or compression exerted by the adjacent material. The more important these forces, the less satisfactory is pure geometrical change as an index of biological activity. Marked departure calls attention to the need for detailed analysis of the acting forces and thus may serve as a valuable indicator of the order of complexity involved.

The following observations may be made: (1) If the density (mass/unit volume) is increasing in a certain region, the specific growth rate of mass must exceed that of volume. If in addition the volume is increasing, or steady, new material is certainly being deposited. (2) If the density is constant, the specific growth rate in volume just equals that of size. If volume is increasing, new material is being deposited; if it is steady, so is the mass; if volume is decreasing, then material is being removed. (3) If the density is decreasing, the specific growth rate in mass must be exceeded by the specific growth rate of volume. If volume is decreasing, then material is in the process of removal.

CONCLUSIONS

An analytical technic is given relating the transformed coordinate method of Thompson to the Huxley-Teissier-Needham procedure for relative growth gradients and is illustrated with Avery's measurements on the growth of the tobacco leaf. Contour lines were used to show regions of similar specific growth in area and crossed lines on the analytical figures indicate the magnitude of the specific maximum and minimum linear growth, which are at right angles to each other.

Isotropic growth is contrasted with isogonic growth and the special case of growth symmetrical with an axis is discussed. The mathematical derivation of the equations used is given.

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Appendix: Mathematical Derivation of the Equations

A. *Basic notation.* If the organism be referred to a set of rectangular coordinates, which conveniently but not necessarily may have its origin at one of the points of the organism, the coordinates (x, y, z) of a moving point may be expressed as a function of time and of their values (x_o, y_o, z_o) at a time chosen as the reference time: $x = x(x_o, y_o, z_o, t)$; $y = y(x_o, y_o, z_o, t)$; $z = z(x_o, y_o, z_o, t)$. That is, each point is earmarked by its position in the reference stage. As growth proceeds, each point moves with a velocity which may be expressed (as in mechanics) in terms of its components $\dot{x}$, $\dot{y}$, $\dot{z}$, in the directions of the axes where $\dot{x} = \partial x/\partial t$, $\dot{y} = \partial y/\partial t$, $\dot{z} = \partial z/\partial t$. Then at any instant each of the quantities $\dot{x}$, $\dot{y}$, $\dot{z}$, is defined at each point of the organism. Henceforth we shall consider them as functions of the coordinates (x, y, z) at the instant under consideration rather than of the coordinates of the reference stage. Thus, for example, $\partial\dot{x}/\partial x$ means the rate of change of $\dot{x}$ with respect to x, the y, z, and t being held constant.

The *direction cosines* of a line are the cosines of the angles the line makes with the x, y, and z axes, respectively. They will be defined by l, m, and n, where l is the cosine of the angle with the x-axis, etc. For a segment of length Δs with one end at the origin and the other at the point $(\Delta x, \Delta y, \Delta z)$, $l = \Delta x/\Delta s$, $m = \Delta y/\Delta s$ and $n = \Delta z/\Delta s$.

B. *Elemental growth-rate in volume per unit volume.* Consider an element of volume in the shape of a rectangular parallelopiped. For convenience choose the coordinate axes so that the origin is fixed at the vertex of the element and the three edges

meeting at that vertex initially lie along the positive halves of the axes respectively, as in figure 1B. Let the lengths of the sides be Δx, Δy and Δz. Then the volume is $\Delta x \cdot \Delta y \cdot \Delta z$.

As growth occurs the element will be deformed. The velocity of the vertex originally at $(\Delta x, 0, 0)$ may be obtained as follows. Due to the choice of the coordinate system, the velocity of the point at the origin is zero. Then the x-component of the velocity at $(\Delta x, 0, 0)$ will be given by $\Delta x \partial \dot{x}/\partial x$ + higher order terms in Δx; the y-component by $\Delta x \partial \dot{y}/\partial x$ + higher order terms in Δx; and the z-component by $\Delta x \partial \dot{z}/\partial x$ + higher order terms in Δx. Then in a short interval dt the coordinates of the point become

$$(\Delta x + \frac{\partial \dot{x}}{\partial x} \Delta x dt, \frac{\partial \dot{y}}{\partial x} \Delta x dt, \frac{\partial \dot{z}}{\partial x} \Delta x dt).$$

Similarly the point originally at $(0, \Delta y, 0)$ moves to

$$\left(\frac{\partial \dot{x}}{\partial y} \Delta y dt, \Delta y + \frac{\partial \dot{y}}{\partial y} \Delta y dt, \frac{\partial \dot{z}}{\partial y} \Delta y dt\right).$$

The point originally at $(0, 0, \Delta z)$ becomes

$$\left(\frac{\partial \dot{x}}{\partial z} \Delta z dt, \frac{\partial \dot{y}}{\partial z} \Delta z dt, \ \Delta z + \frac{\partial \dot{z}}{\partial z} \Delta z dt\right).$$

Thus the rectangular element is deformed into a non-rectangular parallelopiped. The volume of the new figure can be computed by standard methods of analytic geometry or vector analysis, and is found to be, to first order terms in dt, $\Delta x \Delta y \Delta z + \left(\frac{\partial \dot{x}}{\partial x} + \frac{\partial \dot{y}}{\partial y} + \frac{\partial \dot{z}}{\partial z}\right) \Delta x \Delta y \Delta z dt$. Thus the increment in volume is $\left(\frac{\partial \dot{x}}{\partial x} + \frac{\partial \dot{y}}{\partial y} + \frac{\partial \dot{z}}{\partial z}\right) \Delta x \Delta y \Delta z dt$, and the *increment in volume per unit volume per unit time* is $\frac{\partial \dot{x}}{\partial x} + \frac{\partial \dot{y}}{\partial y} + \frac{\partial \dot{z}}{\partial z}$. If Δx, Δy, Δz and dt be made vanishingly small, the neglected higher order terms also vanish, and the above expression thus is the exact expression for elemental increase in volume per unit volume per unit time. In vector analysis it is known as the *divergence of velocity function*, and is denoted by $\nabla \cdot \mathbf{v}$ where $\mathbf{v}$ is the velocity vector.

In the case of growth in a plane the third term is zero, and the expression becomes that for *increment in area per unit area per unit time* (area specific growth-rate of section III): $\frac{\partial \dot{x}}{\partial x} + \frac{\partial \dot{y}}{\partial y}$.

C. *Elemental growth-rate in length per unit length.* Consider a segment of length Δs whose end points may be taken as

(0, 0, 0) and $(\Delta x, \Delta y, \Delta z)$; in this case it is convenient to take Δs, Δx, Δy, and Δz as the changing values of the quantities rather than the fixed values of the particular instant under consideration. Then $(\Delta s)^2 = (\Delta x)^2 + (\Delta y)^2 + (\Delta z)^2$;

$$2\Delta s \frac{d\Delta s}{dt} = 2\Delta x \frac{d\Delta x}{dt} + 2\Delta y \frac{d\Delta y}{dt} + 2\Delta z \frac{d\Delta z}{dt};$$

$$\frac{1}{\Delta s}\frac{d\Delta s}{dt} = \frac{\Delta x}{(\Delta s)^2}\frac{d\Delta x}{dt} + \frac{\Delta y}{(\Delta s)^2}\frac{d\Delta y}{dt} + \frac{\Delta z}{(\Delta s)^2}\frac{d\Delta z}{dt}.$$

Since the origin is fixed at one end of the segment, $\frac{d\Delta x}{dt} = \frac{\partial \dot{x}}{\partial x}\Delta x + \frac{\partial \dot{x}}{\partial y}\Delta y + \frac{\partial \dot{x}}{\partial z}\Delta z$; $\frac{d\Delta y}{dt} = \frac{\partial \dot{y}}{\partial x}\Delta x + \frac{\partial \dot{y}}{\partial y}\Delta y + \frac{\partial \dot{y}}{\partial z}\Delta z$; $\frac{d\Delta z}{dt} = \frac{\partial \dot{z}}{\partial x}\Delta x + \frac{\partial \dot{z}}{\partial y}\Delta y + \frac{\partial \dot{z}}{\partial z}\Delta z$. Substituting these equations in the expression $\frac{1}{\Delta s}\frac{d\Delta s}{dt}$, and using the expressions for the direction cosines of the segment, the result can be written:

$$\frac{1}{\Delta s}\frac{d\Delta s}{dt} = l^2\frac{\partial \dot{x}}{\partial x} + lm\frac{\partial \dot{x}}{\partial y} + ln\frac{\partial \dot{x}}{\partial z} + ml\frac{\partial \dot{y}}{\partial x} + m^2\frac{\partial \dot{y}}{\partial y} + mn\frac{\partial \dot{y}}{\partial z} + nl\frac{\partial \dot{z}}{\partial x} + nm\frac{\partial \dot{z}}{\partial y} + n^2\frac{\partial \dot{z}}{\partial z}.$$

If now the chosen value of Δs be made vanishingly small, the initial direction of the segment being unaltered, the neglected higher order terms in Δx, Δy, Δz become vanishingly small and the expression on the right of the above equation is the exact expression for *elemental growth in length per unit length per unit time* (the specific growth rate in length). Denote this quality by L.

Since L involves the direction cosines l, m, and n, it follows that the specific growth rate in length may in general vary with the direction from the point which is being considered. Thus a small sphere centered at the point and growing for a short time would not in general remain a sphere, but would be somewhat deformed. To study the distribution of values of L, let $L = 1/r^2$, $rl = X$, $rm = Y$, $rn = Z$, and substitute in the equation for L. The resulting equation is that of a central quartic surface in X, Y, and Z, the radius of the surface being r. (In case L is negative, so that r is imaginary, take $L = -1/r^2$.) The properties of these surfaces are well known. When r has a maximum, L has a minimum and vice versa.

In the case of growth in a plane, the expression becomes

$$L = \cos^2\theta\frac{\partial\dot{x}}{\partial x} + \cos\theta\sin\theta\frac{\partial\dot{x}}{\partial y} + \sin\theta\cos\theta\frac{\partial\dot{y}}{\partial x} + \sin^2\theta\frac{\partial\dot{y}}{\partial y},$$

where $\cos\theta = l$ and $\sin\theta = m$. This equation was used in obtaining the specific growth rates in length in section III.

To determine the directions of maximum and minimum rates for plane growth, differentiate L with respect to θ, equate the result to zero and solve for θ. Calling the resulting values of the angle θ_m, it follows that $\tan 2\theta_m = \left[\frac{\partial\dot{x}}{\partial y} + \frac{\partial\dot{y}}{\partial x}\right] \Big/ \left[\frac{\partial\dot{x}}{\partial x} - \frac{\partial\dot{y}}{\partial y}\right]$. Since $\tan 2\theta_m$ has a period $\pi/2$ in θ_m and since values of θ_m corresponding to maximum L must alternate with those corresponding to minimum L, it follows that the directions of maximum and minimum L are at right angles to each other. Necessary conditions for L to be constant with respect to θ are those making $\tan 2\theta_m$ indeterminate; then $\frac{\partial\dot{x}}{\partial y} + \frac{\partial\dot{y}}{\partial x} = 0$; $\frac{\partial\dot{x}}{\partial x} - \frac{\partial\dot{y}}{\partial y} = 0$. Evidently they are sufficient. They are, of course, the Cauchy-Riemann conditions that the complex quantity $\dot{x} + i\dot{y}$ be an analytic function of $x + iy$.

If L is a constant at a point in three-dimensional space, it must be constant in each plane parallel to the coordinate planes; then the Cauchy-Riemann equations must hold in each such plane. Consequently, necessary conditions for constant L are $\frac{\partial\dot{x}}{\partial x} = \frac{\partial\dot{y}}{\partial y} = \frac{\partial\dot{z}}{\partial z}$; $\frac{\partial\dot{x}}{\partial y} + \frac{\partial\dot{y}}{\partial x} = \frac{\partial\dot{y}}{\partial z} + \frac{\partial\dot{z}}{\partial y} = \frac{\partial\dot{z}}{\partial x} + \frac{\partial\dot{x}}{\partial z} = 0$. Evidently the conditions are also sufficient.

There is a simple relation between the growth rate in length per unit length and the growth rate in volume per unit volume at a point. Let L_x denote the value of L in the direction parallel to the x-axis, L_y the value in the direction parallel to the y-axis and L_z the value in the direction parallel to the z-axis. Then $L_x = \partial\dot{x}/\partial x$, $L_y = \partial\dot{y}/\partial y$, $L_z = \partial\dot{z}/\partial z$, and the volume rate $\nabla \cdot \mathbf{v} = L_x + L_y + L_z$. Since $\nabla \cdot \mathbf{v}$ is invariant with respect to a rotation of the axes (as is known from vector analysis) it follows that the volume rate equals the sum of the linear rates in any three mutually perpendicular directions.

D. *Technic of analysis of the tobacco leaf.* The methods used in applying the general formulae to Avery's drawings of the tobacco leaf are not sufficiently general to warrant detailed description. They will be sketched briefly, as suggestive of procedure for similar cases.

In place of calendar time (which was not available to us) the length of the central axis included between the extreme cross markings was chosen as the time variable. This procedure invalidated direct comparisons between rates in different stages, but did not affect ratios between rates in the same stage, or the comparison of such ratios for different stages. It was found that the coordinates of the moving points (intersections of the lines of the network) were very nearly linear functions of this time variable. Slight variations from linearity were observable, but the limitations of the data, mentioned above, made unjustified the extra labor of taking the variations into account.

Each coordinate of each point in turn was graphed against the time variable, and the slopes of the resulting lines were taken as the quantities $\dot{x}$ and $\dot{y}$. These quantities were next represented as functions of the coordinates (x_o, y_o) of the first stage. Then the values $\dot{y}$ for points along the central axis were plotted against the initial distance of the points from the origin, which was taken at the intersection point on the axis nearest to the base of the leaf. The slope of the resulting curve at each point was the value of $\partial\dot{y}/\partial y_o$ at the point. In a similar manner the quantities $\partial\dot{x}/\partial x_o$, $\partial\dot{x}/\partial y_o$, $\partial\dot{y}/\partial x_o$ and $\partial\dot{y}/\partial y_o$ were determined. The slopes were measured with a Tangentmeter.

In the initial stage the quantities $\partial\dot{x}/\partial x_o$ etc. were identical with $\partial\dot{x}/\partial x$, etc. required in the growth-rate formulae. Direct substitution in the formulae was therefore made to determine at each point the growth-rate in area per unit area, and the directions and values of maximum and minimum growth-rate in length per unit length.

For determination of $\partial\dot{x}/\partial x$ etc. for later stages it was necessary to solve linear equations giving the quantities in terms of $\partial\dot{x}/\partial x_o$, etc. The equations were fairly simple, due to the linear relations between the coordinates and the time variable. The resulting $\partial\dot{x}/\partial x$, etc. for each stage were then substituted in the general growth-rate formulae.

The contour lines for growth-rate in area per unit area were located by determining the intersections of each contour with each line of the network, by interpolation between adjacent points of intersection of the network; the points thus determined were joined by smooth curves.

GEOGRAPHICAL VARIATION AND RACIAL STRUCTURE OF ARGYNNIS CALLIPPE IN CALIFORNIA

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INTRODUCTION

SEVERAL species of butterflies in the western part of North America present excellent material for the study of the origin and composition of geographical races as well as for an interpretation of the genetic and ecologic significance of racial or subspecific variations. *Argynnis callippe* (Lepidoptera: Nymphalidae) is a butterfly of wide distribution. It is also very abundant from the point of view of number of individuals per population. These populations are partially (or completely) isolated one from the other by areas more or less large in which breeding conditions are unfavorable. The species is also relatively non-migratory, individuals tending to stay in the region of their early growth. These factors, as well as a great climatic variability in the region, seem to have been responsible for an immense variety of geographical differences between the populations. No attempt will be made to give a complete systematic account of this group as the time and funds have not been available for tracing the complete history of names and types. A systematic account is worthless without this information. Instead, the emphasis will be placed upon the description of variation and distribution which really is greatly needed in this common and highly variable group, since this group has not yet received organization into a logical biological arrangement. In a special section suggestions as to use of names is given. Difficulties for the taxonomist in determination of specimens are to be expected in a group such as the Argynnis, where all species appear so much alike and without good characters for designation and

comparative description. Parallel variation between species is an abundant feature of the Argynnis as in the closely related Melitaea and other butterflies. This feature makes, for the systematist, great difficulties. Different species will appear more like one another in the same region in color and size characters than the members of the same species in different localities (Hovanitz, 1941). Only by means of a dynamic evolutionary concept, a concept of varying gene frequencies in populations and of ecologic population mechanics does it seem possible that the variation of this species can be understood in its fullest. These populations are not uniform; they are not to be expected to be so. It is deplorable that some systematists shun the variant specimen, and eliminate it from their series because it does not follow exactly the description of their type individual.

The variation of most native species of butterflies in California is best studied by relating the variability to the topographic and ecologic features of the country. California may be described roughly as a large central valley about 450 miles long and about 60 miles wide, bordered on the west by a coastal series of ranges split into small valleys and hills, bordered on the east by the very high Sierra Nevada range, closed on the north by a connecting range and closed on the south by a curvature of the lengthwise ranges. This, of course, is only a rough description but will serve to illustrate the main features of butterfly distribution, the means of origination and the maintenance of the differentiation into local and extensive geographical races.

For the type of description which is to follow it would be foolhardy to attempt to adhere to a commonly accepted view that the type locality of a species or race or subspecies is the center of its origin or the center of its distribution. As is now becoming increasingly evident, the type locality is nothing more than the place where the first collector happened to find the different looking individual to which was given a new Latin name. Such a

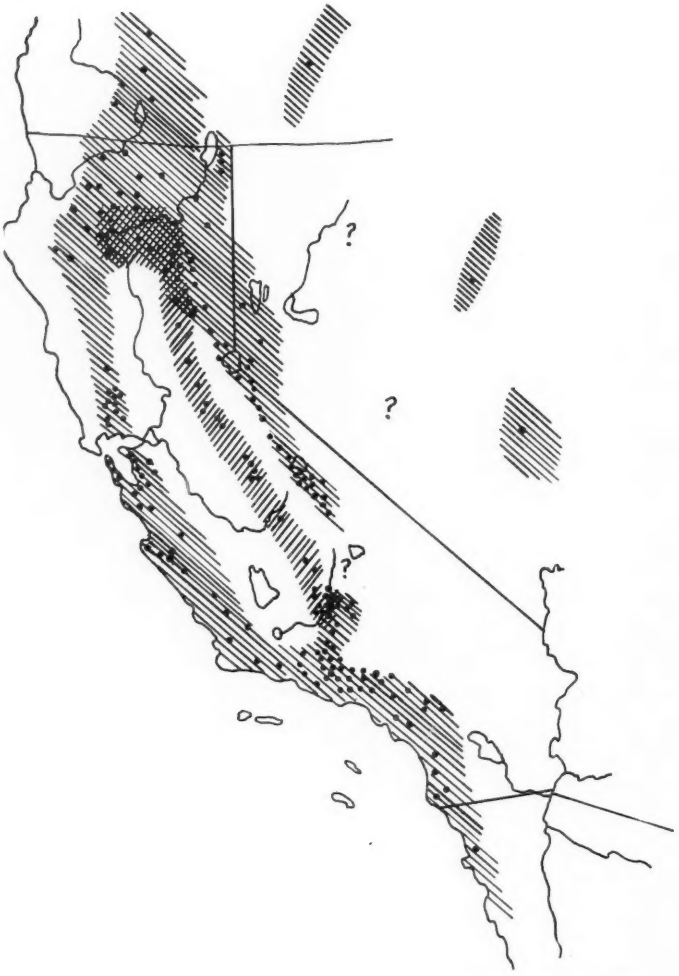

FIG. 1. Map showing the distribution of the characters silvered spots (shading slanting up to the left), and cream-colored spots (shading slanting up to the right). Areas unshaded represent regions from which the butterfly is unreported, and for the most part this means it is absent. Question marks mean that no search has been made to determine the presence of the butterfly in this area. This map also shows the distributions of buff

locality may have been and often was on the zone of intergradation between two variation gradients where hybridization and the intermixing of different genes had produced a very heterogeneous population. Many old names are, therefore, to-day difficult of application, especially where the type series contained individuals now known to belong to two or more geographic races. Such zones of intergradation are very common and are of immense importance for use in evaluating the interrelationships of geographical races. They are, however, difficult to correlate with names.

South Coast Range Populations[1]

Fortunately, the type locality of the individuals upon which the name *callippe* was based forms a very convenient starting point from which to describe the distribution; it is at the end of a geographical gradient where a topographical barrier appears to prevent intergradation with the next closely related gradient. San Francisco, at the end of the arm of land separating the San Francisco Bay from the Pacific Ocean, is one of the most northern localities known for the variation gradient which extends from this place, Berkeley and Mt. Diablo through the south coast range, coastal southern California and into Lower California. This gradient is characterized primarily by a light-colored band (without distinct boundaries) running down the central portion of all wings.

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coloring on the under side of the wings, for cream-colored spots and buff-coloring seem to be related, as also, silvered spotting and yellow coloration. The dots represent known localities of the butterfly. These have not been reproduced on the successive maps. To synthesize character-combinations of a population in any given place it is only necessary to mentally superimpose each of these first five maps over one another. Then for general intensity of coloration, map six can be consulted. Local variation of various types may also serve to affect the results obtained from the general maps, but this should not be excessive. An unknown population can thus, within reasonable limits, be hypothetically determined.

[1] The description of the variation should be followed by reference to the maps which are arranged according to character. For specific localities, more detailed maps not shown here must be consulted.

(See Hovanitz, 1941, Fig. 4.) An occasional individual does not show this. The gradient is not truly a straight line of variation because it has width as well as length; variation occurs from east to west across the coastal ranges as well as north and south along them. At San Francisco (formerly) and San Mateo County this gradient reaches its maximum of darkness. This darkness is produced by a widening of the pattern elements of melanin, by a heavier scaling of melanin-colored basal scales on all wings and by a darkening of the yellow-brown pigment (Group Two-a, Hovanitz, 1941). At Berkeley, 15 to 20 miles inland, under a lesser influence of the coast, the darkening is not as extreme, though occasional individuals are quite dark. South from North Berkeley Hills (Contra Costa Hills), in the San Leandro Hills, the populations become increasingly lighter in color. One particular population east of Hayward has surprisingly light-colored individuals. In between, in East Oakland (Sequoia Park) there are intermediates. Farther south toward Sunol and Calaveras Valley the butterflies are likewise very light in color. It is possible to correlate this color change with the cooling produced by the ocean breezes and fog at Berkeley opposite the Golden Gate, together with a decrease of this climatic effect south through the hills where the San Mateo Ranges and the Santa Cruz Mountains serve to block off the ocean cooling from the interior. At Mt. Diablo, the butterflies are also very pale in color and in size are quite small. This locality is 40 miles inland and far from much fog-cooling. At Carmel Valley, the Santa Lucia Ranges and down to the Santa Inez Ranges the populations are quite variable, depending upon the local climatic conditions. In the interior of the Santa Lucia Ranges occurs one of the lightest colored populations—almost white, but this is continuous with others which are quite dark in color. In the region, Santa Inez Mountains to the San Gabriel Mountains, the populations tend to have a higher percentage of individuals partially or totally lacking the

central light band. This increases east through the Tehachapi Range, where connection is made with the

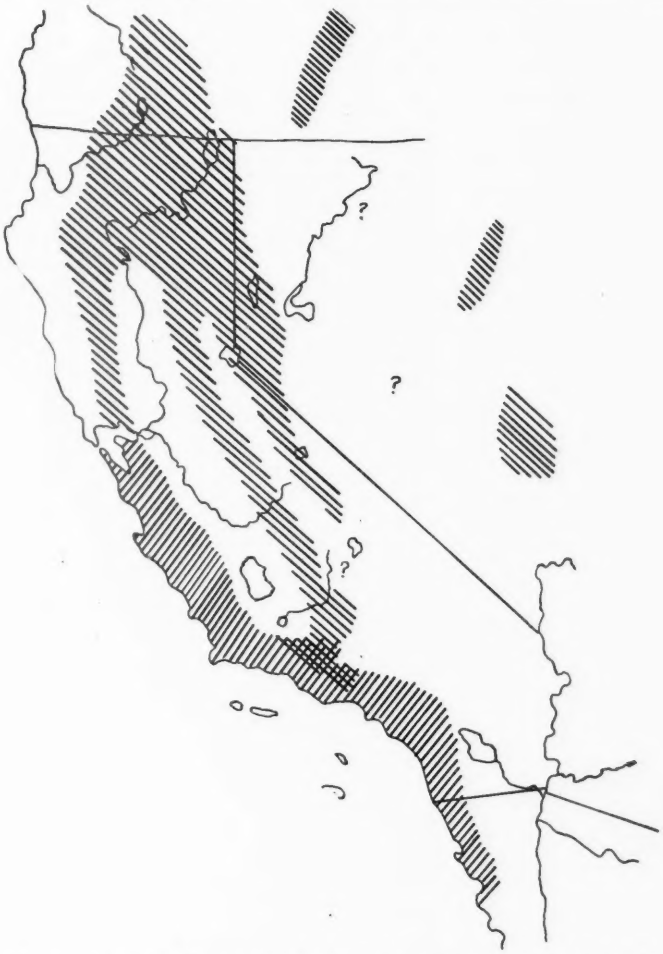

FIG. 2. Map showing the distribution of the light-colored band on the upper surface of the wings (shading slanting up to the right) as compared with a uniformly colored red-brown upper surface.

Sierra Nevada races, which are to be considered later. South in the coastal hills of Southern California and

Lower California there is as much local variability as is present in the San Francisco Bay area, but here the blacker individuals appear to be found in the foothills of the San Gabriel Range. This is likewise true with respect to some Melitaea (*M. chalcedona* especially) and may be correlated with the colder winters and higher rainfall along the western face of this range. No *Argynnis callippe* has ever yet been taken east of the Southern California mountains in the Mohave and Colorado Deserts proper or high in these mountains. It is likewise completely absent from the flat valley areas of the Great Valley of California, from the San Francisco Bay-Santa Clara Valley flat areas, the Livermore Valley, the Salinas Valley, the Carrizo plains and adjoining arid hills, the Santa Maria and Cuyamaca valleys, the valleys and plains of Southern California (Los Angeles Plain, San Gabriel Valley, Santa Clara River Valley, San Fernando Valley, San Bernardino Valley, etc.), being found only in the hilly country—and only locally there. It was perhaps absent from these places even before the cultivation induced by present civilization destroyed the native vegetation. In many places, it is rapidly becoming exterminated by destruction of Viola by the turning over of the soil and now is found only in rocky places for the most part.

Characteristic points concerning the pattern and color of the *callippe* are listed as follows: (1) Silver spots always present on underside hind wings and apex of fore wings; (2) brown scaling between median row of spots on hind wing and base of the wing, no green scaling present; (3) yellow band between marginal spots and median row of spots wide and clearly distinguishable; (4) light-colored band across all wings on upper side; (5) areas on the upper side of the wings, immediately above the silver spots, tend to appear light in color, though this is true more of some populations than of others.

It should be mentioned that dark- or light-colored populations are found along this distributional area anywhere

at (it seems) climatically suitable areas. There appears in this way similar-appearing, completely unconnected local populations separated from one another by other different populations. Climatic selection would appear to be the active agent in the origination or evolution of the local differences and possibly also of the larger series of variations.

Western Sierra Nevada Populations

This coastal gradient connects with the western Sierra Nevada variation gradient through the Tehachapi Mountains. It will be best to describe the Sierra Nevada gradient in general first, before describing the specific state of the zone of intergradation. In the area from the northern Sierra Nevada and southern Cascade Ranges (region west of Lassen Peak and the "Juba" Mountains) to the southern Sierra Nevada the butterflies are characterized by a fairly uniform upper-surface yellow-brown color with a lack of the light band present in the coastal gradient. Also, the spots on the under surface of the wings are not silvered but rather are cream or buff-colored. The band between the marginal spots and the median row of spots is also buff-yellow or just buff instead of yellow as in the coastal gradient. The areas on the upper surface of the wings above the silvered spots are not light colored. The areas between the spots other than the above band on the under side of the hind wing are brown scaled but apparently more uniform than in the coastal gradient. That is, the coastal material has a tendency to have the area interspersed with yellow scaling. Superimposed upon this general description is a variation in darkness and lightness of all the pigmentation from north to south respectively. Melanin pigmentation is increased wherever present, in the width of the pattern elements and in the scaling of the wings at the bases. The brown pigment of the underside, the yellow-brown pigment of the upper side as well as the intensity of the yellow-buff is increased or darkened in the north as compared with the south. The gen-

eral elements remain the same, however. The life-zone preference of the insect in the western Sierra Nevada is the area between the Upper Sonoran and the Transition, ranging from elevations in the north (Shasta, "Juba" Mountains) from 2,000–3,500 feet to 4,000–5,500 feet in the south (Greenhorn Mountains). Through the length of the territory from the Greenhorn Mountains (one of the southern ends of the Sierra Nevada) to the "Juba" Mountains area, the gradient is almost a perfect line with little width. The Central Valley of California is a barrier to intergradation to the west and the boreal regions of the Sierra Nevada are a barrier on the east. Only to the north, in the vicinity of the Yuba River and thence to the Shasta country is there possibility of much movement eastward; here the mountains are relatively low. Passes are not far above the limit reached by the butterflies' preferences as to climate, and, in part, the semi-desert area of the Great Basin invades the volcanic lands of Lassen and Modoc Counties. Here the species is found continuously from the Central Valley foothills of California through to the Great Basin. This is the country where the Basin territory is partly drained by the Pit River, one of the few outlets through the mountain barrier. Phylogenetically, this low opening permits of the exchange of genes from the Basin populations with the coastal or western ones. The effect of this on the variation in the region is great and will be described a little later.

Southern Zone of Intergradation

It remains to describe the connection of the southern end of the range of the western Sierra Nevada gradient given as the Greenhorn Mountains with the coastal gradient in the region where the Sierra Nevada meets the coastal ranges. This is done in a series of steps across the Piute Mountains, the Tehachapi Mountains and the Sierra Madre Range. From the Santa Monica Mountains on the coast, where the typical *callippe* of this region

lives, it is found that in going inland (Charlton Flat, Mint Canyon, Bouquet Canyon and "Ridge Route") the lightness of all colors increases. The butterfly becomes

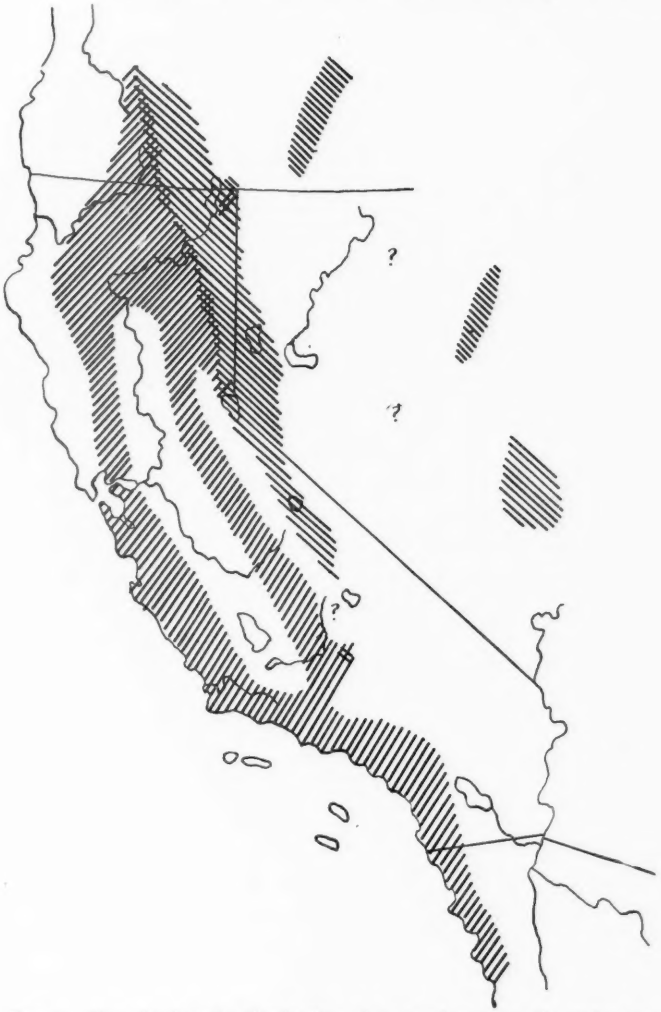

FIG. 3. Map showing the distribution of green pigment on the under surface of the wings exclusive of the marginal band (shading slanting up to the left), as compared with the lack of the pigment.

smaller and the light-colored band and spots on the upper surface of the wings tend to become obliterated, leaving a more uniformly colored wing surface such as is present in the western Sierra Nevada gradient. However, the yellow-brown color is very much lighter than in the latter and the band on the under side of the hind wings is still yellow; the spots are still always fully silvered. The tendency toward these conditions is the more marked the farther from the coast and the farther into the Tehachapi Range the populations exist. In the Tehachapi Range, the butterflies are very lightly colored and the band on the upper surface of the wings is rare; the spots are still silvered. At Havilah, Piute Mountains, the population consists of some silvered, some unsilvered and some intermediate spotted individuals (this is the type locality of *macaria* Edws.); the exact frequency of these types is not known, but there is a high percentage of silvered and unsilvered present. In the Piute Mountains, the first sign of a segregation into an eastern type and a western type of variation is observed. At Kelso Valley, on the eastern side, the tendency is toward silvered spots (23 silvered and two intermediates obtained); whereas on the western side at Havilah, unsilvered are very common, though comparative figures are not available. At Kelso Valley, a tendency toward the appearance of green coloring on the under side of the hind wings is apparent in a few individuals. The relation of this to the distribution of the Great Basin form *nevadensis* will be discussed later. In the Greenhorn Mountains, the segregation into a silvered population on the eastern side of the summit and an unsilvered one on the western side is decidedly apparent, though mixing occurs toward the south, where the populations unite. At Cedar Creek (5,000 feet) on the western side of the summit (7,000 feet) the distribution of variation in a small series was: 10 not silvered or very slightly so, 8 intermediate and 4 well- or fairly well-silvered. On the opposite side of the range at an elevation of 5,500 feet, 14 silvered, two intermediates and no

unsilvered were obtained.[2] These numbers are not large, but they are suggestive of a segregation into distinct populations here, even though the distance between the localities was not more than ten miles airline, and they are separated by a *callippe*-uninhabited area very small in extent. Of course, it can not be assumed that such difference could long exist without a powerful selection for the types or, more certainly the case in this instance, a flow of genes from the centers of populations of each of the silvered and unsilvered forms into this zone of intergradation. Thus the gap is bridged between the western Sierra Nevada gradient and the coastal populations in the south. It should be noted that the gene frequencies have not changed at the same place. Silvering changed at the Greenhorn Mountains; north were unsilvered-gene bearing populations, south the silvered-gene bearing populations. The upper surface pattern made its change in the area from the Tehachapi Mountains to the Bouquet Canyon or the Santa Susana Hills area. In addition, it is observed that the general coloring from dark to light made its changes from north (Shasta and Lassen) to south (Tehachapi) gradually and with no apparent relation to the other changes. It would appear that the genes affecting these characters segregate independently of one another and do so in different geographical regions. However, later we shall see that there is a possibility that silvering and buff coloration are in some way directly related. These both change in the Greenhorn Mountains.

The Northern Populations

As has been mentioned, the northern Sierra region is low, allowing genes to be transmitted between the Basin populations and the western Sierra Nevada populations. The former populations have silvered spots, as do populations farther north in Oregon. Consequently, the re-

[2] The collections at Cedar Creek and eastern side Greenhorn Mountains as well as those at Kelso Valley, Piute Mountains were made on the same day. Much more material from other years is available from the Greenhorn Mountains but is not segregated into specific localities.

gion is mixed, with populations tending to have unsilvered spots on the areas bounding the valley and increasing silvering north and east. This mixture has led to great confusion in the names given to forms from this region. As the Basin populations have a lighter general coloring than the western populations, there is also a transition of this variability through here. The northern limit of the unsilvered populations appears to be the Shasta, Trinity and Siskiyou region. South in the North Coast Range, north into Oregon, east into the Great Basin, silvering is the rule. Only south into the western belt of the Sierra Nevada does unsilvering extend. South through the North Coast Range as far south as Sonoma, Napa, etc., the distribution of this form occurs. The general ground color here is reddish-brown, quite dark in the north (*rupestris*) but becoming lighter to the south (*liliana*). The arms of the San Francisco Bay, the San Pablo Bay and the Carquinez Straits seem to isolate this gradient from the northern end of the south coast gradient, which reaches its northernmost locality in the Berkeley Hills. Some populations in Napa County appear to have individuals seeming to be intermediates (Huichica Creek); the relative abundance of these is very low. This gradient from the Siskiyou, Humboldt and Trinity Mountains to the San Francisco Bay may be characterized then by: (1) a quite uniform red-brown ground color without the light band and spots typical of the south coast form; (2) all heavily silvered spots; (3) a relatively dark color of melanin, red-brown and yellow pigment. There is slight north-south variation in darkness. This gradient has in common with the south-coast gradient the silvered spots, and the yellow colored band on the under side hind wings. It has in common with the western Sierra gradient, the uniform upper surface ground color and the reddish-brown tint to this color rather than the yellowish-brown tint as with the south coast gradient. It should be noted that the only characters changing in the region of the northern end of the valley between the Sierra Nevada

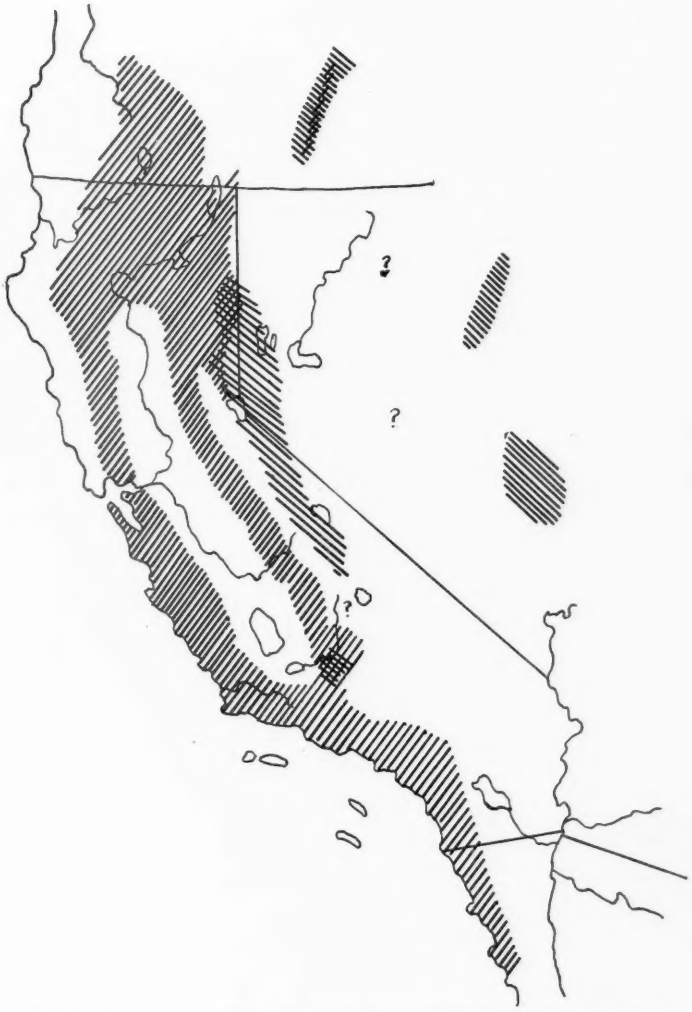

FIG. 4. Map showing the distribution of brown pigment between the spots on the under side of the hind wings (shading slanting up to right) as compared with the lack of this pigment.

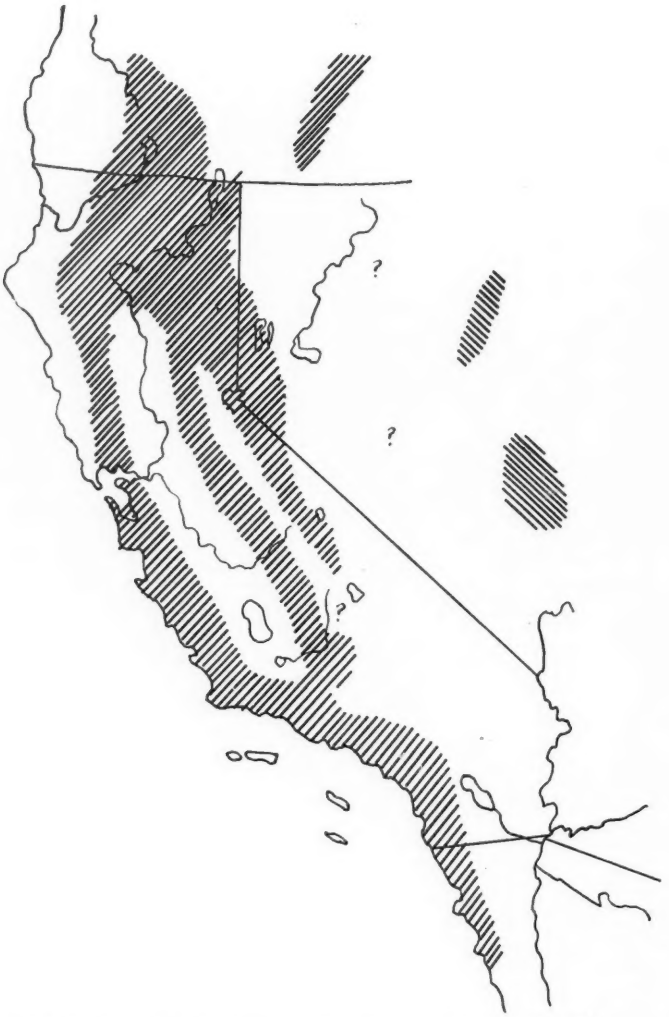

FIG. 5. Map showing the distribution of green pigment scales which occur in the yellow bands on the under side of the hind wings (shading slanting up to the left) as compared with the lack of this pigment.

and the north coast gradients is the change from unsilvered in the former to silvered spots in the latter, and the change from buff pigment on the underside to yellow, respectively. Information as to what happens from here north into Oregon is scanty.

Going eastward through the low mountains in the northern Sierra Nevada, the populations become increasingly silvered (Downieville, etc.). The Great Basin populations (*nevadensis*) are entirely silvered. These populations in California are characterized usually by an upper surface coloration much like western Sierra Nevada populations, that is, with an absence of the central light band and having a uniform ground color; this coloration is fairly light and the melanin is not very extensive. The band on the under side of the hind wings is yellow, not buff, as is the rest of the light pigment on the under side of the wings. In the northern populations, desert Oregon, Modoc and Lassen Counties, there is present brown pigment between the spots on the under side of the hind wings. This decreases southward and appears to be absent from at least Virginia City, Nevada, south through more than ten known localities to Bishop, Inyo County, California. In all populations of this area, the wing is also suffused with green scaling on the same part of the wings. This green is present likewise around the basal side of the border silver spots and on the apex. In the north both the brown and green is present; in the south the green only, on a yellow ground. The yellow band on the hind wing appears to be constant only along the western-most of the Great Basin populations. Eastward, it is suffused with green in most cases (*meadii*). As has been previously mentioned, between the populations of the Basin form and the western Sierra Nevada form, a zone of intergradation occurs. Here, the populations become silvered eastward and do so before they obtain the green on the underside. Thus, areas are formed in which the butterflies are identical with those from a totally unrelated region, namely, the north Coast Range.

It would not appear likely that the populations at these two places, isolated from each other in this way, could have had a common origin. Instead, it seems more plausible to assume that by chance a similar combination of these color genes has taken place. If so, there is parallel evolution in two different places. The circumstance noted, that the point of change-over from silvering to non-silvering seems to bear no relation to the place of change-over from green scaling to non-green scaling, is additional information to show that the genes involved in the production of these geographical races are for the most part free to be synthesized into any combination whatever. Although localities for the green-under-side form are at present unknown south of Bishop on the eastern escarpment of the Sierra Nevada, the appearance of slight green pigment on a few individuals from the Piute Mountains, Kern County, leads one to think that there may be a connection. However, since the locality of the latter is on the edge of the desert (Kelso Valley) it may be a new development of the genes in the population, with no relation to the northern, probably unconnected, form.

SUMMARY OF THE VARIATION AND DISTRIBUTION

Table 1 shows the distribution of these characters in given geographical areas.

It may be observed that the segregation of characters between these different regions is, except for one case, completely independent. This one case is that previously mentioned, non-silvered spots and buff coloration which is present (shown by minus sign) in the western Sierra Nevada gradient. This correlation may be due to linked genes, to a common physiological-developmental basis controlled by a single gene, or possibly to preference selection by a similar type of environmental agency; the first possibility seems the most remote, because a linkage of this sort should break down often enough to give many exceptions. The second seems most probable to the author. Whatever the developmental reason, the two

TABLE 1

	silvered spots present	light-colored band on upper side; also, often light above spots	yellow band on under side as contrasted to buff	brown pigment between spots	green pigment present	green pigment present in yellow band on under side	names applicable to the type of variation or region
South Coast Range gradient	+	+	+	+	−	−	*callippe, comstocki*
North Coast Range gradient	+	−	+	+	−	−	*liliana, rupestris*
Western Sierra Nevada gradient	−	−	−	+	−	−	*juba, inornata, laurina, macaria*
Sierra Madre-Greenhorn Mountains zone	+	−	+	±	−	−	*macaria, comstocki*
North Sierra Nevada intermediate zone	+	−	+	+	−	−	*juba, rupestris, inornata, liliana*
Modoc-Lassen and north	+	−	+	+	+	−	*semivirida, laura*
Eastern Sierra Nevada	+	−	+	−	+	−	*nevadensis*
Eastern Great Basin	+	−	+	−	+	+	*meadii*

characters seem to be present together in the same individuals as well as being present in the same general geographic region.

As noted before, two regions have a similar distribution of characters, namely, the north-coast range gradient and the intermediate north Sierra Nevada zone. Since the ground color of the latter is relatively light or intermediate brown in color, most butterflies from this region can not be distinguished from butterflies from the southern part of the north-coast range gradient (Napa and Sonoma Counties) without a long series. In the latter case, the aberrant individuals serve to differentiate the population; the aberrant individuals are more like their nearest relatives (in the intermediate north Sierra Nevada zone aberrancies are taken with a little green coloration and with silvering tending to disappear). In the southern part of the North Coast Range gradient, aberrancies tend to get a light band across the upper surface of the wings—like typical *callippe*.

"Darkness and Lightness"

Besides the six apparently qualitative color variation types described above, which might be related to six (or five) specific genes, there is the type of variation which controls the darkness and lightness of all pigments on the insect, that is, the quantity of it. Genes controlling this type must act in development at such a time as to affect all pigments, that is, must be general influencers of pigment metabolism or deposition. The characteristics of the variation in this butterfly are an increase in intensity and area of the melanin pigment (Group 1), an increase in intensity of the red-brown or yellow-brown pigment (Group 2a) and likewise of the yellow pigment where present (Group 2b). The melanin increase in area comes from a widening of the pattern elements and a heavier scaling extending out from the base of the wings. Because of the light-colored band which is present in the South Coast Range gradient the specimens from this area are difficult to correlate with material from elsewhere which lack it. The map (Fig. 6) shows the distribution separately. It should be noted that this variation for the most part is independent of the other variation. It also seems to show a more direct correlation to specific environmental influences (see Hovanitz, 1941, for full data). The darker individuals and populations are present in regions of least light intensity, greatest moisture or humidity, lowest temperature and longest available growth period. The lighter occur under the opposite conditions. It is interesting that the populations at the north end of the South Coast Range gradient possess a much greater amount of melanin pigment than the populations at the southern end of the North Coast Range gradient. Since the South Coast Range gradient has a light-colored band (of Group 2a pigment) on the wings, it may be that the darkening of the wings can not take place with that pigment so long as the given gene is present and the color change then takes place in the melanin pigment (Group 1 pigment). North of the San Francisco Bay, the inhibitor

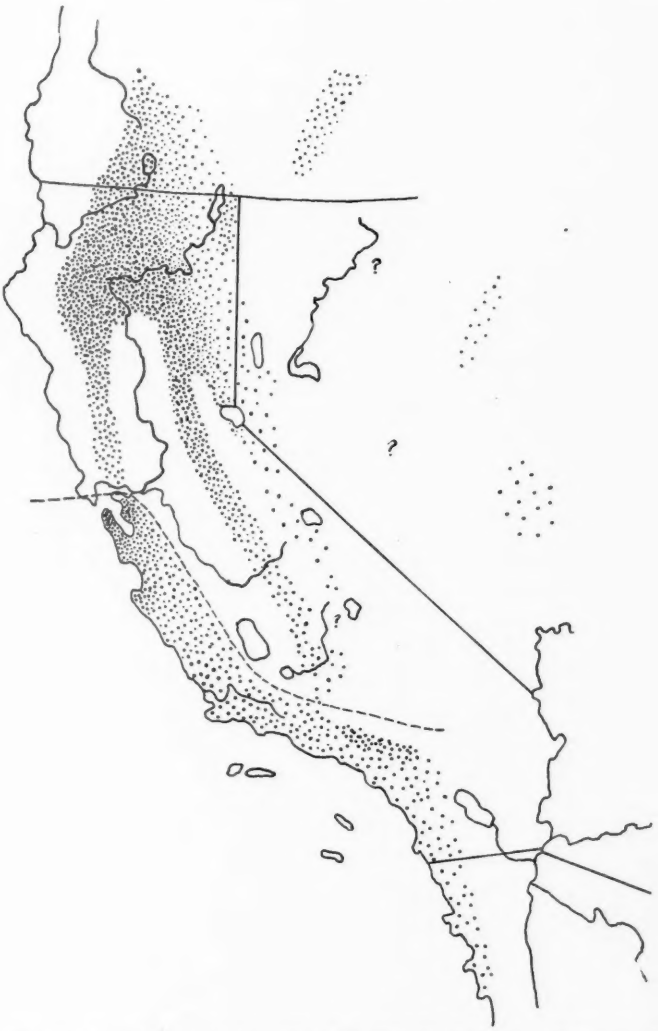

FIG. 6. Map showing by intensity of shading the darkness or lightness of the upper surface of the wings. The area above and to the right of the dashed line should be considered separately from that below and to the left because the light-colored band existent in specimens from the latter region interferes with the comparison between these regions.

gene (which controls the light-colored band) is not present; the Group 2a pigment is darker and the Group 1 pigment is lighter, it appears, to make up for this deficiency.

OTHER ECOLOGICAL CORRELATIONS

The significance, if any, of silvered and unsilvered spots on the under surface of the wings is quite incomprehensible. In this species, as well as others in the genus, there seems to be no significant correlation between silvered spots or unsilvered spots and environmental factors. In general, but rather weakly so, silvering seems to be commoner in more southern regions than northern ones in the subfamily to which the Argynnis belong. Perhaps the coloration is correlated with the buff pigmentation above mentioned and carried along with it. But in this case, the buff coloration might have some significance.

The significance of the light band across the wings of the South Coast Range gradient possibly may be correlated with the southern location of the region. It has the most southern distribution in the species and would be expected to be light in color. Whatever the physiological developmental basis and foundations of the color differences, it might appear that any gene that controls a process in ontogeny that allows the animal to be better adapted to live in the environment of the south, will be selected for. Apparently such genes are correlated with a lighter color (Hovanitz, 1941). By means of several different methods, as earlier mentioned, a lighter color could have been obtained in this region, but apparently sufficient change was not possible of accomplishment by a continuation of the North Coast Range gradient. Such a relationship is not unknown in other species for example, *Coenonympha typhon california* is very light in color in this same approximate region and dark elsewhere.

The significance of the green pigment on the under surface of the wings is not too easy to correlate with any

specific environmental conditions. Other species in the same general region (Great Basin-Rocky Mountains), have such a coloration in this region which is found nowhere else in North America, namely, *Argynnis bischoffi-eurynome*,[3] *Argynnis edwardsi*, and possibly one other whose name is doubtful. Whether this coloration is of any physiological significance, directly or indirectly, with any ecologic factor is hard to imagine. The country is characterized especially by cold winters, hot and dry summers, and light-colored terrain.

The significance of the brown pigment between the spots on the under side of the hind wing is correlated with climatic conditions, as was discussed with the general pigment changes (Group 2a, Hovanitz, 1941). The absence of the pigment is found in arid, hot and southern regions, etc.

The names applied to this variation of *Argynnis callippe* in California are:

callippe Boisduval 1852. *Ann. Soc. Ent. Fr.*, 21: 302 (San Francisco, California).

comstocki Gunder 1925. *Entom. News*, 36: 8 (Los Angeles).

macaria Edws. 1877. *Field and Forest*, 3: 86 (Havilah, Kern Co., Calif.).

laurina Wright 1905. Butterflies West Coast, 138 ("Southern California Mountains").

inornata Edws. 1872. *Trans. Am. Ent. Soc.*, 4: 64 (Downieville, Calif.).

juba Bdv. 1869. *Ann. Soc. Ent. Belge*, 12: 60 ("Juba mountains").

laura Edws. 1879. *Can. Ent.*, 11: 49 (Nevada).

rupestris Behr 1863. *Proc. Calif. Acad. Nat. Sci.*, 3: 84 (Sierra Nevada).

liliana Hy. Edws. 1876. *Proc. Calif. Acad. Sci.*, 6: 170 (St. Helena, Napa Co.).

nevadensis Edws. 1870. *Trans. Am. Ent. Soc.*, 3: 14 (Virginia City, Nev.).

semivirida McD. 1924. *Can. Entom.*, 56: 42 (Aspen Grove, B. C., Canada).

meadii Edws. 1872. *Trans. Am. Ent. Soc.*, 4: 67 (Turkey Creek Junction, Colorado).

It is the author's suggestion that these names might be used in the following manner, it being understood that the types have not been examined:

[3] For some unknown reason, *bischoffii* is considered a race of *eurynome* in the latest check list. *Bischoffii* appears to be the oldest name available here: *Argynnis bischoffii* Edws. 1871. (1870), *Trans. Am. Entom. Soc.*, 3: 189, *Argynnis eurynome* Edws. 1872, *Trans. Am. Entom. Soc.*, 4: 66. The hyphened name is used here to designate the general species, since *bischoffii* is not well known.

Argynnis callippe callippe for the gradient extending from Berkeley and San Francisco on the north to Lower California on the south along the coast ranges. (*comstocki* as synonym).

Argynnis callippe laurina or *rupestris*, for the gradient extending from the mountains at the head of the Great Valley to the Greenhorn Mountains, *i.e.*, the unsilvered race. (*laura, laurina* or *rupestris, juba, macaria, inornata* being smaller entities if used at all).

Argynnis callippe liliana Hy. Edws., for the gradient extending from Northern California to San Francisco Bay in the North Coast Ranges.

Argynnis callippe nevadensis for the triangular gradient extending from British Columbia on the north to Bishop on the south, and eastward to the Front Range of Colorado.

These are major divisions, but if it is desired that smaller and easier-to-use units be organized the following is suggested:

Argynnis callippe callippe as above, bearing in mind the tremendous local variation which also might be further subdivided. In the latter case *comstocki* could be used for the south or many other names might be applied for local populations.

Argynnis callippe macaria for the heterogeneous mixture extending from the Sierra Madre to the Greenhorn Mountains. This is in some places quite constant and is intermediate between the western Sierra Nevada gradient and the coastal gradient.

Argynnis callippe laurina for the light-colored, southern individuals of the western Sierra Nevada unsilvered gradient.

Argynnis callippe inornata for the intermediate-colored silvered part of the western and northern Sierra Nevada zone.

Argynnis callippe rupestris for the area around the northern end of the Great Valley where the coloration is fairly uniform except for the mixing of the unsilvered and silvered.

Argynnis callippe liliana for the gradient extending from the zone designated *rupestris* to the San Francisco Bay. There is a change in darkness and lightness of ground color here.

Argynnis callippe nevadensis for the zone extending from Virginia City, Nevada, and Truckee, south to Bishop in which green is present and brown absent between the spots on the under side hind wings, and a yellow band is present.

Argynnis callippe semivirida for the zone extending from Lassen County, California, to British Columbia along the eastern fringe of the Cascade Mountains. The characteristic here is green and brown pigmentation on the under side hind wing. It should be remembered that this grades completely into *nevadensis*.

Argynnis callippe meadii for the Great Basin zone proper in which the yellow band is suffused with green scaling for the most part.

Some systematists may feel it desirable to extend further the analysis of the variations by applying names to even more restricted local populations. The author

does not think this necessary or desirable, but does consider it of importance that records of variations and distribution be published. The author believes the important part to be the variation and the biological significance of the variation, and this can be studied in any number of ways. For practical purposes of identification in a collection it is desirable that local populations be given names, but variation considered "unnamable" is often as important as, or more important than the latter and should receive its proper place in publications.

Material Examined

The following list shows by region the approximate quantity of material examined by the author. Detailed listing of localities, numbers, etc., was considered to be too bulky for reproduction. San Francisco Bay area, except northern part, ± 700 individuals; Santa Lucia Mountains—Ventura area, ± 200; Ventura—Lower California area, ± 500; Sierra Madre—Greenhorn Mountains area, ± 400; southern to northern Sierra Nevada on west side, ± 60; southern to northern Sierra Nevada on east side, ± 400; southern Oregon and northern California, ± 300; north Coast Range area, ± 150; Steens Mountains, Oregon and eastern Nevada, ± 25; total of about 2,750, much of this being in the zones of intergradation and samples of specific, pertinent populations.

In addition to this, confirmatory data in the literature may be added. The author has personally collected in and become ecologically acquainted with populations in all the regions shown on the maps, with the exception of eastern Nevada. The area unshaded on the maps, except for those parts marked with a question mark, almost certainly harbor no population of this species, most of this area having been extensively investigated.

Conclusions

The author believes that the above data warrant the conclusion that geographic races or subspecies are units composed of populations having more or less similar

genes. The differences between races may be ascribed to different combinations of these genes existing in different populations which may be synthesized into any combination. Theoretically, any race may be formed with the proper genetic materials; in practice, this would be of considerable difficulty, since innumerable generations may be needed for selection to provide the proper "genetic environment" for the new gene combination. It is concluded that for the most part racial characters are either directly or indirectly adaptive (also the conclusion of Dice, 1940). It would not be fitting to formulate any definite conclusions on the origin of species from these data. However, authors seem to do so consistently on no more information. It should be noted that the term species is being used in much recent literature for units no larger than the smallest units suggested in this paper as possible races or subspecies. Finally, it may be concluded that subspecies or races are purely units of convenient classification.

ACKNOWLEDGMENTS

The most sincere thanks must be given to the following for their great generosity in allowing the use of their collections or material for the purpose of studying the geographical distributions. Without this courtesy, much of the completeness of the maps would have been lost and definite knowledge of some of the intergradation zones might have been left in doubt: M. L. Walton, C. N. Rudkin, J. A. Comstock and the Los Angeles Museum staff, the entomology staff of the California Academy of Sciences, J. E. Cottle, C. P. Medlar and the San Diego Natural History Museum, Lowell Hulbirt, J. W. Tilden, W. Finley and C. D. Michener.

SUMMARY

The geographical distribution and variation of *Argynnis callippe* in California and adjacent regions is described with a genetic viewpoint by which it is assumed that populations are different because they have different

genes or different frequencies of genes. Maps are given which show that only two of six distinctive characters are always completely correlated, the distribution of the others bearing no definite relation to the others. Additional data are given to support the conclusion reached in a former paper that most characters distinguishing geographic races are adaptive in character, but that this adaptiveness is generally of a concealed nature.

NOTE. Since the above was in process of publication the following important information has been obtained. The gap in geographical distribution between the southern and northern parts of the north Coast Range has been partially filled by material from Comptche, Mendocino County, California. This population would be located on the map directly northwest of the northernmost denoted dot in the southern part of the north Coast Range (fig. 1, opposite the first bulge of the coast north of San Francisco Bay). Therefore, the range of the species extends closer to the coast in this region than was originally expected. In the characters shown by these specimens, which number twelve, the maps represent accurately the character combinations as hypothetically determined earlier. One specimen represents an intermediate condition between silvered and unsilvered spots (fig. 1), the farthest south in the Coast Range that such material is known. Verification of expected character combinations of this sort serves to show that one can predict population analyses nearly as accurately as one can predict results in the more experimental sciences. This material was examined and made known through the courtesy of C. D. Michener and L. P. Grey.

LITERATURE CITED[4]

Dice, L. R.
1940. AM. NAT., 74: 212–221.
Hovanitz, W.
1941. *Ecology*, 22: 259–284.

[4] The general evolutionary literature is not discussed in this paper because ready reference can be made to certain very recent and excellent general evolution treatises or to the author's paper cited where a bibliography is given.

THE EFFECT OF ZINC SULFATE UPON AN INBRED POPULATION OF DROSOPHILA MELANOGASTER[1,2]

DR. DONALD GREIFF

Introduction

The present study was undertaken to determine the effect of zinc sulfate upon the population characteristics of a highly inbred strain of *Drosophila melanogaster*. Preliminary tests showed that the introduction of zinc sulfate into a synthetic medium caused a deleterious differential in the emergence of imagoes. The flies were inbred by brother and sister matings for eleven generations before the three following lines were established:

a. The control line; inbred flies raised upon the control medium.
b. The selected line; inbred flies raised from the twelfth to the thirty-second generation upon the test medium.
c. The selected-control line; inbred flies raised from the twelfth to the twenty-fifth generation upon the test medium and from the twenty-sixth to the thirty-second generation upon the control food.

Representative numbers of flies of the twelfth, sixteenth, twenty-fifth, twenty-sixth and thirty-second generations were tested for population characteristics as regards: fecundity;[3] productivity; time of imaginal emergence, the duration of time from mating to the emergence of adult offspring; weight of imagoes; inherent vitality.

[1] From the Department of Biology and the Department of Immunology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Md.

[2] The writer wishes to express his appreciation to the late Dr. Raymond Pearl for suggesting the present problem and his thanks to Dr. Roscoe R. Hyde for his generous help and criticism.

[3] In order to avoid the confusion which has grown up in the literature, the terms "fecundity," "productivity" and "fertility" used in this report are those introduced by Hyde (1914b), who first brought to light the factors involved in the determination of fertility in this species. These were defined as follows: (1) Fecundity—the number of ova or sperm produced by an adult organism. (2) Productivity—the number of offspring arising from a single mating. (3) Fertility—the ratio between the number of eggs laid and the number of offspring issuing therefrom expressed in per cent.

Materials and Methods

The ancestral flies employed in this investigation were obtained from Professor T. H. Morgan in December, 1919, by Dr. Raymond Pearl, of happy memory. They were of the Old Falmouth strain of *Drosophila melanogaster,* long inbred in Morgan's laboratory. Pearl and Parker (1922) further inbred this stock and from it raised their "107" line. This line was kept as a laboratory stock by pedigree breeding for several years and then carried along by mass brother and sister matings. The parental pair of the present study was made from a single brother and sister mating. This line was continued by brother and sister matings for eleven generations, from which the three lines of the present investigation were established. Thereafter the flies of the same generation and line were grouped and randomly selected males and females were used as the parents of the succeeding generation.

The control medium used for the cultivation of the flies was a modification of the synthetic medium, S-101, developed by Pearl, Allen and Penniman (1926). Preliminary experiments with serial dilutions of various compounds showed that 10^{-3} gram of zinc sulfate per gram of synthetic medium acted as the best selective agent. Therefore, the test medium was formed by adding 0.10 gram of zinc sulfate to 99.90 grams of the control medium. The culture bottles were kept in a standard double-walled, biological incubator. The temperature was maintained at $25 \pm 0.3°$ C. by means of a bi-metallic thermoregulator. Humidity was accurately controlled by a saturated ammonium chloride (c.p.) solution as described by Obermiller (1924) and reported to maintain a humidity of 79.3 per cent. at 25° C. (International Critical Tables, 1926).

Flies, twelve hours old, were used in determining the duration of life without food. Each fly was put in an isolation tube and placed in the incubator. The technique used in handling the flies in the incubator was that de-

veloped by Powsner (1935): (1) clustering the isolation tubes as near as possible to the thermoregulator; (2) rotating isolation tubes randomly in the incubator; (3) keeping number of isolation tubes constant.

Fly weights were made upon a chainomatic balance. It was found that the greatest sensitivity of the balance occurred between .003 and .005 of a gram. The weights of five flies, 12 hours old, fell within this range. Therefore, to obtain the greatest accuracy in weight, the imagoes were weighed in groups of five. In order to obtain an exact measure of fecundity, it was necessary, after the total emergence of imagoes had been counted, to determine the number of undeveloped reproductive units. The medium within the culture bottle was melted and poured into several glass plates edged with rubber weather-stripping to act as a retaining wall. The culture bottle was washed with hot water to remove any eggs, larvae and pupae therein. The washings were added to the previously poured medium. After drying at room temperature, the plates were placed upon a modified bacterial colony counting apparatus and the unhatched eggs, dead larvae, non-emerged pupae enumerated. This method differs from that of Pearl (1932) and Alpatov (1932). Traumatizing of the various units is reduced to a minimum; 50 cc of medium is used instead of a few cc; the counts are made after a fairly long period of time and not, as the other techniques required, at the end of 24 to 72 hours. Thus this technique allows the cultures to approach the normal conditions of the laboratory.

EXPERIMENTAL DATA

The life spans of the several generations were as follows:

12th generation—February 1 to February 24.
16th generation—April 2 to April 27.
25th generation—July 25 to August 16.
26th generation—August 6 to August 28.
32nd generation—November 3 to November 28.

Fecundity

Table I gives the statistical constants for the fecundity of the generations tested. It is apparent that the introduction of zinc sulfate into the medium did not affect the fecundity of the females for several generations. Thus it

TABLE I

THE STATISTICAL CONSTANTS FOR THE FECUNDITY PER BOTTLE PER EIGHT MATED DAYS OF THE CONTROL, SELECTED AND SELECTED-CONTROL LINES

Generation	Control line		Selected line		Selected-control line	
	Mean (eggs laid in 8 days)	No. of bottles	Mean (eggs laid in 8 days)	No. of bottles	Mean (eggs laid in 8 days)	No. of bottles
F_{12}	370.62 ± 58.71	8	306.54 ± 33.58	11		
F_{16}	326.11 ± 59.01	9	313.81 ± 37.73	11		
F_{25}	328.78 ± 24.62	14	205.72 ± 37.91	11		
F_{26}	267.18 ± 16.40	16	142.21 ± 17.64	14	136.30 ± 21.01	13
F_{32}	191.93 ± 23.98	15	273.00 ± 9.92	14	199.37 ± 14.58	16

would appear that the mode of action of the agent used was different from the mode of action of the environmental factors employed by Delcourt and Guyénot (1911), Guyénot (1913a, 1913b, 1913c, 1913d), Richardson (1925), Hanson and Ferris (1929), Pearl (1932) and Alpatov (1932).

The technique of this research was such that egg laying over a limited time was measured. Thus the lowering of fecundity of the selected line admitted of three explanations: (1) the change in fecundity was due to a change in the germ plasm; (2) the change in fecundity was due to a loss of certain portions of the germ plasm; (3) the change in fecundity was due to a change in the physiological cycle of egg-laying and the greatest amount of eggs was laid after the eight-day period was passed. Hanson and Ferris (1929), Shapiro (1932), Alpatov (1932) have shown that in *D. melanogaster* the greatest number of eggs is laid in the first ten days. The foregoing was also shown for *D. pseudoobscura* by Dobzhansky (1935).

If the first alternative is correct, it would mean that simultaneous mutations took place in a large number of

flies in a fairly short time. To account for the rise in fecundity of the selected line and selected-control line between the 27th and 32nd generations would mean that a reversal to the initial status of the germ plasm occurred. The experience of many investigators with the rate of mutation makes the tremendous mutation rate necessary for the first alternative extremely remote.

It was found that the change in fertility of the 12th and 16th generations between the selected line and the control line was due to the loss of larvae in the selected line. If this loss of certain larvae meant the loss of germ plasm or of certain genes contained therein, the fecundity of the 16th generation would have been expected to be significantly less than the fecundity of the control line. However, this was not found to be the case and therefore the second alternative seems unlikely.

The fecundity of the control line and the selected line were not significantly different for the 12th and 16th generations. By the 25th generation the fecundity of the selected line was significantly less than that of the control line. It must be remembered that the amount of zinc sulfate imbibed by a single fly during its larval stage is extremely small. Therefore, it is possible that the zinc sulfate had no effect for the first several generations upon fecundity because the amount present was not great enough to influence the normal physiology of the female in this regard. However, by the 25th generation the concentration of zinc sulfate within the flies may have been such that it could act upon the function of egg-laying. By the 32nd generation, however, the flies could have developed a tolerance to the reagent present and returned to the normal cycle of egg-laying. In the selected-control line the return to the normal cycle may be accounted for by the dilution of the zinc sulfate present to such an extent that it no longer acted physiologically upon egg-laying. Thus it would seem that the change in fecundity was due to an upsetting of the normal cycle of egg-laying.

The downward trend exhibited by the control line was not significant. The fecundity ranged from 370.62 to

191.93 eggs per bottle per eight mated days. This is in keeping with the figures found by Hyde (1914a, 1914b, 1914c, 1914d), Shapiro (1932) and Alpatov (1932). Thus the results obtained by the technique used in this study are in keeping with the figures obtained directly by other investigators.

Productivity

Table II contains the statistical constants for the emergence of imagoes. There is no statistically significant difference in the emergence of male and female ima-

TABLE II

STATISTICAL CONSTANTS FOR IMAGINAL EMERGENCE OF THE CONTROL, SELECTED AND SELECTED-CONTROL LINES.

Generation	Control line		Selected line		Selected-control line	
	Mean (Imagoes per bottle)	No. of bottles	Mean (Imagoes per bottle)	No. of bottles	Mean (Imagoes per bottle)	No. of bottles
F_{12} ♂♂ + ♀♀	241.12 ± 35.21	8	156.72 ± 17.65	11		
♂♂	121.62 ± 19.03	8	77.90 ± 8.96	11		
♀♀	119.50 ± 16.51	8	78.81 ± 9.13	11		
F_{16} ♂♂ + ♀♀	171.88 ± 32.81	9	114.54 ± 14.57	11		
♂♂	83.55 ± 14.91	9	61.54 ± 6.61	11		
♀♀	88.33 ± 18.09	9	53.00 ± 8.49	11		
F_{25} ♂♂ + ♀♀	211.42 ± 18.89	14	114.27 ± 24.48	11		
♂♂	105.14 ± 8.61	14	59.63 ± 12.06	11		
♀♀	106.28 ± 10.46	14	54.63 ± 12.75	11		
F_{26} ♂♂ + ♀♀	177.37 ± 15.18	16	90.42 ± 13.85	14	90.15 ± 16.72	13
♂♂	87.00 ± 7.40	16	48.92 ± 7.52	14	44.69 ± 8.19	13
♀♀	90.37 ± 8.06	16	41.50 ± 6.71	14	45.46 ± 8.73	13
F_{32} ♂♂ + ♀♀	137.60 ± 24.28	15	204.00 ± 8.73	14	142.93 ± 16.07	16
♂♂	70.13 ± 12.97	15	103.53 ± 5.28	14	69.62 ± 8.03	16
♀♀	67.46 ± 11.50	15	100.00 ± 5.05	14	73.31 ± 8.40	16

goes of the several lines. This was contrary to the experiences of Hyde (1941a), Warren (1918) and Lawrence (1940). The foregoing investigators found that the ratio of emergence favored the females. The results obtained for this investigation were not unexpected, however, for the samples used for any one test were too small to show the existence of a sexual differential in emergence.

That a relationship exists between fecundity and productivity there can be little doubt. Castle, *et al.* (1906),

Moenkhaus (1911) and Hyde (1914c) demonstrated that close inbreeding did not diminish the productivity of *D. melanogaster* provided productive pairs were selected to continue the stock. This fact was again confirmed in the present investigation, although the control line exhibited a slight downward trend in productivity between the 12th and 32nd generations, and the selected line between the 12th and 26th generations. Since the samples used were small and the mean differences relatively small, it was felt that the means of the individual lines were randomly distributed.

The lower mean productivity of the 12th and 16th generations was due to the elmination of potential imagoes during the larval stage. The decreased productivity of the 25th and 26th generations was due to the lowering of the fecundity for those generations as pointed out in the preceding section. Thus the actual numbers of component units in the control, selected and selected-control lines were in the same ratio. With the return of the selected and selected-control lines to normal fecundity in the 32nd generation, the productivity of the lines also returned to normal. Therefore, it is apparent that the fertility of the flies was immediately affected by the presence of the zinc sulfate in the medium. By the 25th generation the fertility had returned to normal but the probable accumulation of the agent within the imago had lowered the productivity by lowering the fecundity. Thus the concentration of zinc sulfate necessary to disturb the fertility of the organism was small and adaptation was soon accomplished. After the tolerance to the zinc sulfate was established, however, it was great enough to buffer the effects of greater concentrations of the reagent.

Time of Imaginal Emergence

Table III gives the statistical constants for the time of emergence of the populations of the several lines following eight-day matings of the parental flies. The differences in the mean time of emergence of the males and

TABLE III

STATISTICAL CONSTANTS FOR THE TIME OF EMERGENCE (FOLLOWING EIGHT-DAY MATINGS) OF THE CONTROL, SELECTED AND SELECTED-CONTROL LINES

Generation	Control line		Selected line		Selected-control line	
	Mean (days)	No. of flies	Mean (days)	No. of flies	Mean (days)	No. of flies
F_{12} ♂♂ + ♀♀	14.61 ± .05	1934	14.98 ± .04	1724		
♂♂	14.64 ± .07	976	14.99 ± .06	857		
♀♀	14.58 ± .06	958	14.98 ± .06	867		
F_{16} ♂♂ + ♀♀	14.12 ± .11	1547	13.57 ± .07	1260		
♂♂	13.92 ± .16	752	13.66 ± .10	678		
♀♀	14.30 ± .16	795	13.46 ± .10	582		
F_{25} ♂♂ + ♀♀	12.41 ± .05	3185	12.24 ± .06	1257		
♂♂	12.49 ± .08	1473	12.21 + .08	656		
♀♀	12.34 ± .08	1712	12.27 ± .09	601		
F_{26} ♂♂ + ♀♀	12.97 ± .06	2835	12.76 ± .06	1266	11.60 ± .06	1175
♂♂	12.95 ± .08	1389	12.73 ± .08	685	11.68 ± .09	584
♀♀	12.99 ± .08	1446	12.80 ± .09	581	11.52 ± .08	591
F_{32} ♂♂ + ♀♀	13.53 ± .08	2067	12.75 ± .03	2879	13.00 ± .06	2288
♂♂	13.49 ± .11	1058	12.86 ± .05	1479	12.99 ± .09	1114
♀♀	13.60 ± .11	1009	12.64 ± .05	1400	13.02 ± .08	1174

females of the control line were not significant. This was also true for the selected line with the exception of the 32nd generation. The females of this generation emerged in a significantly shorter time than did the males. No significant difference was found in the selected-control line.

The combined sexes, males separately, and females separately of the control line seemed to have a cyclic trend. The time of emergence was greater during the winter months than during the summer months. The longest time of emergence for this series was the 12th generation, and the shortest time of emergence was at the 25th generation. It is a well-known fact that the physiological processes of an organism vary greatly during the course of a year. This is especially noteworthy when the organism, with a cycle in its natural habitat, is brought into the laboratory. In this regard it has often been found that even when the environmental conditions are controlled for experimental purposes, the seasonal variation often persists. Thus Schneider (1940b) working with *Tribolium confusum* reported that the length of larval period was longest in October and November,

shortest in June and July. He concluded that the spring and summer broods developed faster than the fall and early winter broods.

Therefore, it is not at all surprising that the mean time of emergence in this investigation displayed a cyclic trend. However, several other facts must be considered. Bliss (1926) found that the higher the temperature, the shorter the time of prepupal development in the males and females of *Drosophila melanogaster*. Bonnier (1926) using a sex-linked mutant yellow stock reported that the mean time of emergence from eggs to imagoes at 25° C was 232.21 hours for the males and 227.98 for the females. At 30° C the developmental times were 187.63 hours for the males and 178.10 hours for the females. Alpatov (1930) working with the wild-type fly reported that the time from egg-laying to pupation required 93.16 hours at 28° C and 200.86 hours at 18.2° C. Thus the results obtained for the present work would have been expected if the temperature at which the cultures were kept had varied simultaneously with the external temperature. However, as was pointed out earlier, the environment surrounding the flies was kept uniform from generation to generation. But it must be recognized that other but less obvious meteorological phenomenon accompany changes in temperature. Thus the cycle may be due to one of two factors: (1) the cycle was due to uncontrolled manifestations present in the atmosphere surrounding the incubators; or (2) the cycle was due to the genetical make-up of the fly. The cycle may also be the resultant of the interaction of both of the foregoing factors.

The mean time of emergence of the selected line for the sexes combined, males separately, and females separately exhibited the same trend as the control line from the 12th to the 26th generation. Instead of continuing its upward swing, as was found for the control line, there was a leveling off and the mean of the 26th and 32nd generations were not significantly different. Therefore, it would seem that the accumulation of the agent in the selected flies

again reached a threshold at which point it caused a change in the normal physiology of the fly. The trend of the selected-control line was like that of the control line for the generations tested. The mean time of emergence of the 32nd generation was significantly higher than the mean of the 26th generation for the sexes combined, for males and females separately. The females of the selected line were also found to be more rapidly returning to the control position than the males, following the removal of the deleterious agent. Thus it would appear (1) that the males are more susceptible to the effect of the agent or (2) a change has occurred in the germ plasm through the use of the agent.

Weight of the Imago

Table IV gives the statistical constants of the weights of five-fly groups. In keeping with other observations on insects, Eigenbrodt (1925) and Schneider (1940a, 1940b, 1940c, 1940d, and 1940e) found that the females were significantly heavier than the males. This held for the several lines and all the generations of this investigation

TABLE IV

STATISTICAL CONSTANTS FOR WEIGHTS OF IMAGOES OF THE CONTROL, SELECTED AND SELECTED-CONTROL LINES (IN TERMS OF GROUPS OF FIVE FLIES)

Generation	Control line		Selected line		Selected-control line	
	Mean (gm.)	No. of groups	Mean (gm.)	No. of groups	Mean (gm.)	No. of groups
F_{12} ♂♂ + ♀♀	.00461 ± .00011	49	.00423 ± .00014	49		
♂♂	.00377 ± .00010	19	.00357 ± .00012	27		
♀♀	.00514 ± .00009	30	.00503 ± .00016	22		
F_{16} ♂♂ + ♀♀	.00414 ± .00008	57	.00445 ± .00009	53		
♂♂	.00367 ± .00007	27	.00396 ± .00008	29		
♀♀	.00456 ± .00010	30	.00505 ± .00008	24		
F_{25} ♂♂ + ♀♀	.00449 ± .00006	101	.00454 ± .00008	69		
♂♂	.00392 ± .00003	51	.00395 ± .00002	37		
♀♀	.00508 ± .00004	50	.00522 ± .00005	32		
F_{26} ♂♂ + ♀♀	.00446 ± .00007	88	.00439 ± .00007	87	.00448 ± .00009	81
♂♂	.00396 ± .00003	44	.00378 ± .00004	45	.00367 ± .00002	39
♀♀	.00528 ± .00005	44	.00504 ± .00005	42	.00522 ± .00005	42
F_{32} ♂♂ + ♀♀	.00419 ± .00009	55	.00433 ± .00006	85	.00425 ± .00012	56
♂♂	.00358 ± .00007	27	.00379 ± .00004	42	.00353 ± .00007	30
♀♀	.00477 ± .00009	28	.00487 ± .00005	43	.00509 ± .00013	26

tested. The control line males seemed to have a cyclic trend in regards to weight. The peak occurred at the 25th and 26th generations, the troughs at the 16th and 32nd generations. Thus the males went through a single cycle in a ten-month period. The control line females also appeared to possess a cyclic trend in weight. The peaks occurred at the 12th and 26th generations, the troughs at the 16th and 32nd generations, Thus the females went through one and one-half cycles in a ten-month period.

The presence of zinc sulfate disturbed the normal cycle in both the males and females of the selected line. The mean weights of the males tended to vary little in the later generations tested, although in the earlier generations the cycle was much like that for the control line males. Thus it would seem that after a certain concentration of zinc sulfate was present within the fly a change in its physiology occurred. This is further shown by the fact that the removal of the reagent from the medium resulted in the mean weights returning to the control position. The physiology of the females was immediately affected by the zinc sulfate. The effect was such that they showed little variation in mean weight from the 12th to the 26th generation. By the 32nd generation, however, an adaptation seems to have occurred and the mean weight was not significantly different from that of the control. The removal of the environmental agent did not change the physiology of the females. From the foregoing it would appear that the zinc sulfate acted differentially on the sexes. The males tended to preserve their cycle but continued breeding on the test medium finally upset this tendency. The females, on the other hand, were affected immediately but with continued breeding tended to return to the normal (control) cycle.

Inherent Vitality of Imago Measured by the Duration of Life in the Absence of Food

Table V gives the statistical constants for the inherent vitality of the newly emerged imago. The mean duration

of life of the females of the control line of this investigation were significantly greater than the mean of the males in each generation tested. This conforms with the findings of Pearl and Parker (1924), who state: "The normal relation between the sexes in respect of mean duration of life (females longer-lived than males) observed under full feeding, is preserved under conditions of complete starvation." Greiff (1940) found that the males of the wild-type fly lived significantly longer than the females under a condition of complete starvation. Hyde (1913)

TABLE V

STATISTICAL CONSTANTS FOR THE INHERENT VITALITY OF THE CONTROL, SELECTED AND SELECTED-CONTROL LINES

Generation	Control line		Selected line		Selected-control line	
	Mean inherent vitality (hours)	No. of flies	Mean inherent vitality (hours)	No. of flies	Mean inherent vitality (hours)	No. of flies
F_{12} ♂♂ + ♀♀	44.55 ± .53	381	46.92 ± .54	334		
♂♂	43.03 ± .77	198	46.10 ± .77	184		
♀♀	46.19 ± .71	183	47.92 ± .73	150		
F_{16} ♂♂ + ♀♀	38.10 ± .54	425	38.58 ± .59	418		
♂♂	34.15 ± .81	205	35.26 ± .80	221		
♀♀	41.78 ± .64	220	42.30 ± .77	197		
F_{25} ♂♂ + ♀♀	26.03 ± .48	345	23.70 ± .49	246		
♂♂	23.82 ± .72	169	22.33 ± .66	144		
♀♀	28.15 ± .65	176	25.64 ± .68	102		
F_{29} ♂♂ + ♀♀	31.82 ± .48	290	32.87 ± .59	242	30.94 ± .47	217
♂♂	30.08 ± .61	148	30.29 ± .76	121	29.77 ± .62	105
♀♀	33.63 ± .73	142	35.45 ± .86	121	32.03 ± .69	112
F_{32} ♂♂ + ♀♀	28.18 ± .60	245	26.56 ± .64	300	29.16 ± .48	386
♂♂	26.23 ± .92	121	24.71 ± 1.01	143	26.58 ± .74	197
♀♀	20.09 ± .75	124	28.24 ± .77	157	31.84 ± .56	189

found the foregoing to be the case with fed imagoes. It is not unlikely that the differences in physiological response were due to the fact that Hyde and Greiff used different strains of flies from those used by Pearl and Parker. This is probable in light of the fact that the flies used in the investigation of Pearl and Parker were the ancestral parents of the flies of this research.

The females of the selected line were longer lived than the males. The differences were significant for every generation excepting the 12th. The differences in mean duration of life of the selected-control line females were

significantly greater than the males. Thus it would seem that the agent used was able to upset the normal relationship of males to females for this strain. However, an adaptation occurred after the 12th generation and the physiology of the fly returned to its normal inherent vitality.

The mean duration of life without food appeared to possess a cyclic trend. It was found, however, that the amplitude of the waves of the cycle was dampened by inbreeding. The control and selected lines behaved similarly. The peaks were at the 12th and 26th generations, the troughs at the 25th and 32nd generations. The zinc sulfate acted as a stimulant on the 12th generation selected males, but by the 16th generation the difference was not significant. The removal of the zinc sulfate did not alter the mean duration of life without food. Thus it would seem that the reagent had little influence on inherent vitality.

INCIDENTAL OBSERVATIONS

The following observations were noted during the course of this study:

(1) The selected line flies required approximately twice the amount of ether and about twice as long to anesthetize as the control line flies.
(2) Anesthetized selected line flies displayed a tetany of the wing muscles.
(3) The control medium possessing control flies was firm for about 20 days; the test medium possessing selected line flies rapidly became loose at the end of three days.

The foregoing also held for the control medium having selected line flies.

SUMMARY

(1) Three lines of inbred populations of *Drosophila melanogaster* were established: (a) the control line composed of flies raised on synthetic medium; (b) the selected line raised on synthetic medium plus zinc sulfate; and (c) the selected-control line raised for several genera-

tions on the test medium and brought back to the control medium.

(2) Representative numbers of flies of several generations were tested for their population characteristics, namely: fecundity, productivity, time of emergence, weight of imago, and inherent vitality.

(3) Preliminary experiments showed the 10^{-3} gram of zinc sulfate per gram of synthetic medium acted as the best selective agent of the several concentrations tried.

(4) The fecundity of the control line was not affected by inbreeding.

(5) The fecundity of the selected line was lowered by the presence of the zinc sulfate but in later generations an adaptation occurred.

(6) The zinc sulfate seemed to delay the laying of the eggs.

(7) The productivity of the flies was lowered by the zinc sulfate, but in later generations this returned to normal, seemingly through adaptation.

(8) The time of emergence from mating to adult offspring showed a cyclic trend.

(9) The control line males and females exhibited a cyclic trend in weight; the males went through a single cycle in a ten month period, while the females one and one-half cycles in the same period.

(10) The zinc sulfate disturbed the weight cycle which was immediately apparent in the males but delayed in the females.

(11) An adaptation occurred in the females in later generations and the cycle for weight returned to normal.

(12) It was pointed out that the various results reported in the literature on inherent vitality was probably due to the use of different strains of *D. melanogaster.*

(13) Duration of life without food appeared to possess a cyclic trend with the amplitude of the waves dampened by inbreeding.

(14) The zinc sulfate had little effect upon inherent vitality as measured by duration of life without food.

CONCLUSIONS

Zinc sulfate introduced into the culture medium reduced the productivity and fecundity of an inbred population of *Drosophila melanogaster*. This reduction was evident for several generations as measured by the control. The effect, however, was not permanent as these functions returned to normal even in the presence of this agent on further inbreeding. Apparently these two physiological characteristics became adapted to the new environment.

There also occurred a sexual differentiation in weight, as the normal cycle was disturbed for both sexes. Continued inbreeding restored the cycle in the females but not in the males at the end of the 32nd generation.

The time of emergence of the imago gave a cyclic trend that was disturbed by the zinc sulfate. This was not restored on continued inbreeding up to the 32nd generation.

The duration of life of the imago in the absence of food possessed a cyclic trend that was not affected by the zinc sulfate.

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THE USELESSNESS OF THE SPINDLE FIBERS FOR MOVING THE CHROMOSOMES

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INTRODUCTION

IN a previous paper (Piza, 1939) it was pointed out that the chromosomes of *Tityus bahiensis* are inserted at the spindle very prematurely, that is, at a time long before the metaphase of the first spermatocyte division. If the chromosomes were provided as usually with a single localized insertion region, then this peculiarity wouldn't be noticed or at least would draw no special attention from the investigator. But, since the chromosomes, as was shown in several papers (Piza, 1939, 1939a, 1940, 1941), are provided with a point of attachment at each extremity, the fact referred to above acquires an enormous significance, permitting the formation of a more or less exact opinion concerning the part played by the spindle fibers in moving the chromosomes.

The outstanding question is simply to decide whether or not the fibrillar elements of the spindle connected with the chromosomes have any influence upon the latter, pulling them toward the poles or merely guiding them while some unknown forces act on their separation.

The theories attempting to explain the movement of the chromosomes are many, and were recently summarized by Schrader (1940). Among these, one—the traction theory or "Zugfaserntheorie" of the German authors—had assumed that the fibers, anchored at the centers and attached at the other end to the chromosomes, could pull the chromosomes to the poles. This theory was criticized mainly because of the fact that the fibers, as they become shorter, never seem to become correspondingly thicker. Thus, it seems clear that the reduction in length

of the fibers can not be attributed to a true contraction effect.

Based on his studies on Tityus, Piza (1939a) again came to describe the filaments of the spindle as effective agents in the movement of the chromosomes, considering them as a material support which the chromosomes have to absorb in order to get to the poles. The mechanism proposed, suggested by a decrease in consistency responsible for the bending of the chromosomes toward the poles, due to the absorption of the spindle material by the point of attachment, seemed to him accountable for the progressive reduction in length of the fibers not accompanied by any increase in diameter.

It is a well-established fact that the capacity of the chromosomes to perform regular anaphase movements is directly associated with their power in producing spindle fibers and that the akinetic chromosomes lose concomitantly both the faculty of moving normally as well as that of originating spindle fibers. Whether or not these faculties depend upon one another is not known. However, the fact that the chromosomes of Tityus are attached at the spindle long before metaphase is reached does modify completely any point of view which considers the fibers as mechanical agents in the anaphase movements, even in the passive manner proposed by Piza.

The Facts

A complete account of meiosis in Tityus will be given in another paper. In the present one only some very remarkable features of the first spermatocyte division will be reported.

When the zygotene stage is finished the nucleus of the spermatocyte appears occupied by thin threads of irregular chromomeric outline each of which is formed by two strands intimately united along their whole length. In its complicated way within the nucleus each thread describes a wide spiral whose segments are separated by very narrow turns. From this stage till metaphase the

chromosomes pass through a continuous process of length reduction, on twisting and surface uniformization, showing more and more clearly their double parallel nature. The process of spindle formation could not be followed. But the spindle is completely set up long before the chromosomes acquire their ultimate metaphase shape. At that time the bivalents, formed by two parallel

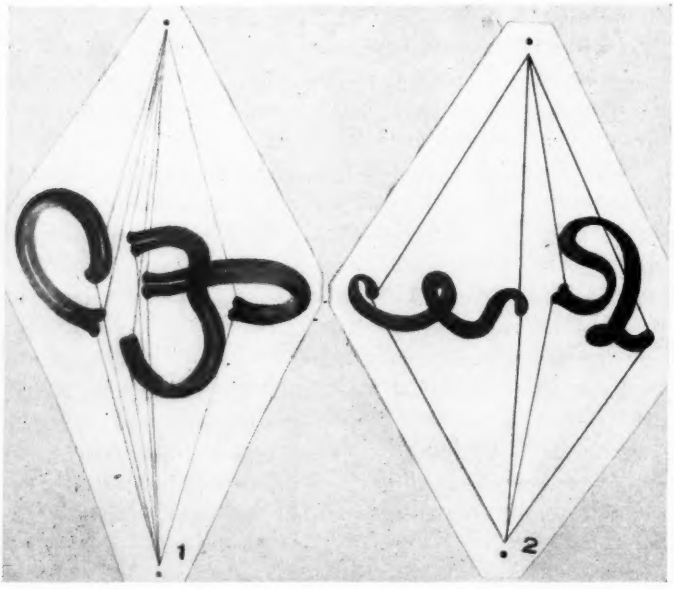

FIG. 1. Tityus chromosomes attached at the spindle long before metaphase of the first spermatocyte division. These chromosomes are far from reaching their ultimate rod-shaped form. FIG. 2. Long spiralized chromosomes of the first spermatocyte division of Tityus already attached at the spindle. In spite of having both extremities connected with the poles by means of spindle fibers, these chromosomes can move and rotate freely in order to assume their ultimate metaphase shape.

threads, are still more or less twisted, have their extremities out of the plane of the equator and are bent in the most varied manner. Moreover, they are already attached at the spindle by their two ends (Figs. 1 and 2).

Finally, becoming shorter the chromosomes finish by being divested of their last spiral twists and by orienting themselves in the equatorial plane, in such a manner that each component of a pair has both extremities directed toward the same pole (Fig 3). This orientation is sometimes accomplished before the chromosomes have reached their final length. The metaphase rod-shaped chromosomes may appear straight or bent. In lateral view many

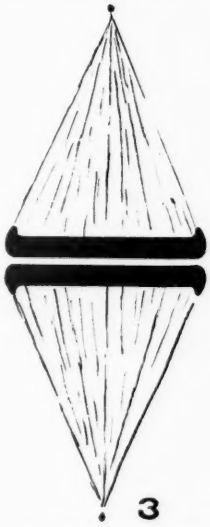

FIG. 3. Typical metaphase I chromosome of Tityus.

very thin fibrillae can be detected along the entire polar side of the chromosomes (Fig. 3). In discussing the significance of the spindle fibers, only those attached at the extremities of the chromosomes will be taken into consideration. In top view the paired chromosomes seem to be already divided, since something like a split gives to the bivalents a quadripartite aspect. However, considering that this particular feature can be analyzed with certainty in no other situation, it has been differently interpreted by Piza (1939a, footnote 15). Some prometaphase figures suggest that the extremities of the

chromosomes anticipate their body in reaching the equatorial plane (Figs. 1 and 2). The chromosomes are held together by a mutual attraction, since no chiasmata can be detected at any stage of meiosis.

DISCUSSION

Since fiber formation precedes anaphase movements and spindle fibers disappear as the chromosomes advance toward the poles, it seems probable that chromosome movements are dependent on the fibers produced by them before they begin to separate. Moreover, as was shown specially by Carlson (1938, 1938a, 1940) in grasshopper neuroblast, akinetic fragments of chromosomes, which do not develop spindle fibers, are also devoid of the capacity of regular orientation, being unable to establish any normal connection with the spindle. On the other hand, all movements prior to separation and even the orientation movements take place without spindle fibers. Thus Schrader (1941) has shown recently that the chromosomes of *Anisolabis maritima* move inside the nucleus toward the part of its wall which corresponds externally to the position of the centrosomes (as happens also in other species in the so-called bouquet stage) and, as the centrosomes separate from each other, the chromosomes, forming two groups, accompany them in their way to the poles.

The instrumentality of the spindle fibers in moving apart the chromosomes appears therefore unnecessary. Indeed, chromosomes which move and orient themselves independently of the fibers do not need to be attached to any special kind of fibrillar elements in order to get to the poles. The behavior of Tityus chromosomes comes in support of such a view. Actually, in this scorpion the spindle is well established when the chromosomes are still formed of long threads intimately united and twisted (Fig. 1). From that time on the chromosomes are seen to be connected with the poles by filaments attached at their extremities. The production of the fibers precedes

not only the occupation of the equatorial plane by the chromosomes but also the orientation of their ends with regard to the poles. When the extremities of the chromosomes are about to take a place in the plane of the equator they are already connected with both poles. Notwithstanding this, in their movement, as they go farther and farther from one pole they approach more and more the opposite one, without altering in any way their fibrillar connection with both. In addition to this, the bivalents are frequently bent in the most varied manner, so that, to assume the characteristic rod-shaped metaphase form they have to perform unwinding movements, which they do easily as if no fibers were present at their ends. The same is observed during orientation. In order to avoid bridge formation, that is, to secure a perfect separation, it is necessary that the chromosomes become completely untwisted so that both extremities of one partner can go to one pole while the extremities of the other go to the opposite one. In doing this the points of insertion have to reverse repeatedly their connection with the poles, looking now to the one then to the other. Finally, to pass from some intricate prophase situations to the very simple metaphase one we are forced to assume that the fibers can cross through one another or even through the body of the chromosomes without being broken or deranged. All this indicates that the spindle fibers do not have any influence in moving the chromosomes. Unfortunately, the present investigation furnishes no information concerning the nature of the spindle fiber itself. But, whatever may be the ultimate nature of the fibers they are to be considered as a component of the spindle continuously produced between the points of insertion and the poles whatever may be the relative situation of these points. They are the inevitable consequence of some unknown forces developed between the poles and each individual point of attachment, as the luminous bundle which crosses the space of a dark room is the consequence of the dust which fills the room and of an orifice

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in the wall giving entrance to the light from the outside. It seems to me that the following assumption may point the way to the solution of this important problem: The fibers stretched from the insertion region of the chromosomes to the poles may well be considered as the result of a very feeble coagulation process due probably to something like an electrical force between these points, which determines the formation of something like a colloidal fibril exactly in the line connecting the two points. If the kinetochore changes its position with regard to the pole, filaments are formed in every new line while the colloidal material entering in the constitution of the preceding ones goes again into the sol state.

Returning to the facts, I do not know how far the results presented here can be generalized. However, there are in the literature at least two cases which are in full agreement with my findings in Tityus. One of these is the case of Protenor, the other the case of Sciara.

In Protenor, as was pointed out by Schrader (1935), the X-chromosome behaves differently in the two meiotic divisions of the spermatocyte. In the first division the X-bivalent lies flatly on the equatorial plane and after separation both chromosomes move parallel toward the poles. In the second division the X-chromosome, which does not divide, takes in metaphase a position perpendicular to the plane of the equator and without modifying that position goes indifferently to one or other pole. What is specially to be emphasized here is the fact that in moving toward one of the poles the X-chromosome of the second spermatocyte division of Protenor maintains a fibrillar connection with both, so that, as it moves, the spindle fibers become progressively shorter forwardly and correspondingly longer backwardly. The chromosome, therefore, moves freely as if no fibers were attached at its extremities.

The Sciara case studied by Metz and collaborators (1926) is equally demonstrative. In the maturation division of the spermatocytes of this fly the ten chromosomes

of the diploid set, without conjugation, put themselves individually in fibrillar connection with a single pole. Then, while six of the chromosomes move toward this pole as in an ordinary mitosis the other four go away from it, to form in the antipolar region a bud in which they are eliminated. What is singularly interesting in Sciara case is the backwards movement of the elimination chromosomes. In fact, these chromosomes, as they are going away from the pole, maintain their point of attachment constantly connected with it by means of spindle fibers. This extraordinary occurrence affords a strong support to the view developed in the present paper.

The very recent investigation of Pease (1941) on pressure effects upon the spindle figure and chromosome movement brought forward valuable experimental data for a more adequate interpretation of the mechanics of mitosis. Subjecting Urechis eggs to different hydrostatic pressures during various stages of the first mitotic cycle, Pease came to the following important results:

(1) Destruction of visible spindle begins with a pressure less than 2,000 lbs/in^2, being complete with a pressure of 3,000 pounds. By 3,000 pounds' pressure no trace of fiber structure is left, but the spindle area remains demarked by granulations slightly orientated longitudinally. With increasing pressures this granulated area begins in its turn to disappear, being entirely absent when the pressure reaches 10,000 pounds.

(2) Chromosome movements are perfectly normal under 1,000 pounds' pressure, are more and more slowed by pressures higher than 2,000 pounds, and more or less completely blocked by 6,000 pounds' pressure.

(3) Under pressures exceeding 2,000 pounds early anaphase chromosomes aggregate in fluid "vesicles" which move as units. While moving apart the vesicles generally remain connected by bridges.

(4) After the release of pressure new functional half-spindles are formed which pick up chromosomes at all mitotic stages from metaphase on.

As it was pointed out above, the spindle fibers are to be regarded as an inevitable consequence of a coagulation process developed between the point of attachment of the chromosomes and the poles. The immediate cause of the coagulation which creates the fibers connecting chromosomes and poles is at the present state of our knowledge entirely unknown. That the spindle fibers do not represent any particular structure of the dividing cell which cooperates effectively in the movement of the chromosomes was emphasized by Gurwitsch (1926). In the opinion of that writer the achromatic figure is a resultant devoid of signification of the forces working in the bipolar field in which it is formed. The spindle then would be the morphological expression of the forces of a bipolarity set up relatively early in the dividing cell, which in the favorable situations may be recognized shortly after the cell enters into mitotic activities. Though Gurwitsch's conception, according to which the chromosomes would be elements arisen *de novo* as a consequence of gelatinization of substances produced within preestablished lines (Bahnen), can not be accepted due to many reasons not discussed here, there is nothing against the idea developed by him that the mitosis may be the result of the establishment of a field of bipolarized forces in the cell. It is true that Gurwitsch left untouched the fundamental question of the primary origin of the bipolarity. But, as soon as this bipolarity is set up, all elements of the cell under the action of the working forces move following the vectors representing those forces. No element of the cell is charged with any special activity. Even the chromosomes are considered as members of a dynamical system moving as a whole. Since no reference is made to the probable kinetic influence of the central bodies upon the chromosomes as well as to the cases in which chromosomes move independently as if they belonged to different minor systems, the theoretical considerations of Gurwitsch offer no ground for a more detailed analysis.

Hughes-Schrader and Ris' (1941) recent investigation has shown that x-ray-induced fragments of the Steatococcus chromosomes behave mitotically like unbroken chromosomes, each fragment being typically oriented at metaphase, producing its own half spindle, and the parallel halves disjoining without lagging at anaphase, through many cell generations. Though rarely, some cases have been recorded in course of Steatococcus investigation, in which chromosome fragments vesiculate at metaphase or anaphase or lag on the spindle without producing spindle fibers. Vesiculated fragments, however, frequently showed autonomous anaphasic separation.

Now, returning to Pease's results we will see that chromosomes can still move at a time when the spindle fibers have disappeared completely. But, at that time the spindle substance is still present in the spindle area as more or less dispersed granules. Whether or not the total suppression of chromosome movement coincides exactly with total disappearance of spindle fiber substance is not clear. What is clearly shown is that the rate of chromosome movement is normal when the spindle fibers are equally normal and becomes more and more slow till complete cessation as the fibers are entering into a more and more pronounced disorganization. With the suppression of the hydrostatic pressure chromosome movement and spindle fibers come again to normality.

Altogether the facts discussed above point to the conclusion that the spindle fibers are nothing else than the optical expression of a physiological activity of the chromosomes, that is, of their power of responding with coordinated movements to the action of the forces operating during mitosis. Or, in other words, the spindle fibers are the consequence of the chromosomes' capacity of moving. Chromosomes or chromosome fragments (Steatococcus case) which move, determine spindle fibers formation, while chromosomes (Vivipara case reported by Pollister, 1939) or chromosome fragments (grasshopper

neuroblast) which have lost the faculty of moving regularly, are unable to produce spindle fibers. In Pease's experiments the chromosomes physiologically altered by increasing pressures lose progressively their reactivity to the moving stimulus from the poles, which in its turn became probably weaker, so that, when the chromosomes reach inactivity the power which determines the formation of a fibrillar gel connecting them with the poles being smaller than the opposite solation power of the hydrostatic pressure, no spindle fibers can be produced.

The point of attachment. A fact well established by the students of the cell is that the chromosomes are universally attached at the spindle by a single point. This point of attachment has received many different names among which primary constriction and kinetochore are the most common. In some cases the presence of a minute deeply staining granule has been noted in the insertion region of the chromosomes. When this granule is absent and the primary constriction obscure the position of the point of attachment is indicated in the long chromosomes by a pronounced repulsion which marks the beginning of their separation, as well as by the shape of the anaphase chromosomes, which, being bent exactly at the point of insertion, form an angle with equal or unequal sides according to the localization of that point.

In addition to the chromosomes of Ascaris germinal lineage whose compound nature was established some time ago, there are in the literature a number of cases in which the behavior of the chromosomes in anaphase suggests new methods of spindle attachment. Among these I wish to discuss here only the three which were considered by the respective authors as truly distinct from the typical cases. These are the case of Tityus (Piza, 1939, 1939a, 1940, 1941), the case of Steatococcus (Hughes-Schrader and Ris, 1941) and the case of the X-chromosome of Protenor (Schrader, 1935).

Tityus case. Tityus chromosomes are considered as undoubtedly provided with a spindle attachment at each

extremity. This is proved (a) by the strong repulsion at both ends clearly observed at metaphase and anaphase of the first meiotic division of the spermatocytes; (b) by anaphase movement in which the chromosomes, having the extremities turned toward the poles, begin to separate in perfect parallelism but assume later an arch-shaped form; (c) by the repulsion at a single extremity in spontaneous fragments and (d) by the similar behavior of the chromosomes at the second division of the spermatocytes.

At the metaphase of the spermatocytes I in which the existence of two kinetochores can be observed in the clearest way, the chromosomes, besides the fibers connecting the extremities with the poles, show in the triangular space formed by these fibers and their body a continuous pellicle of striated appearance. Since the beginning of my work on the scorpion I considered this structure as a pellicle and not as row of independent fibers because in oblique view of the chromosomes the fibrillar elements seem to belong to something like a very delicate film. This pellicle (if my interpretation is correct) is individual, that is, each chromosome possesses its own, so that each half spindle is seen to comprise three independent pellicles joined by their apices at the centrosomes.

The spindle, generally speaking, presents in fixed material a fibrillar aspect due most probably to a gelification of substances in a bipolarized field of forces. (See for discussion Piza, 1939a.) In this report, only the fibers attached at the kinetochores and due to an effect proceeding from them have been considered as true spindle fibers.

In Tityus only the fibers derived from the extremities of the chromosomes have a predetermined and constant point of origin. All other fibrillar elements seen along the body of the chromosomes seem to have neither a determined point of insertion nor any constancy in number. Consequently, I did not have any solid ground for interpreting these fibrillae other than as a secondary product of the coagulation of the material enclosed in the triangular field occupied by them.

The idea that Tityus chromosomes may be provided with a linear series of kinetochores as the compound chromosomes of Ascaris or with a diffuse spindle attachment as suggested by Schrader (1935) in the case of Protenor and by Hughes-Schrader and Ris (1941) in Steatococcus is contradicted by all facts presented above in favor of the existence of two terminal kinetochores.

With so many concrete arguments in support of the existence of a kinetochore at each extremity, the assumption that other points of attachment exist, suggested only by the presence of fibrillar elements along the entire length of the chromosomes, would be unnecessary since these elements could be interpreted as due to the coagulation of the material contained within the fibers of the extremities and the body of the chromosomes.

Piza (1939a) has considered the spindle as being homogeneous. The fibers connecting the chromosomes with the poles would result from the coagulating activity of an enzyme put out by the points of insertion. Any other fibers which would appear there by means of natural or artificial (due to fixation) processes would follow the lines of force of the spindle and could adhere to the body of the chromosomes laid across their way. These fibers would differ from the terminal ones in having no fixed point of insertion. The enzyme to which the gelification of the spindle was attributed would determine the formation of a fiber following exactly the line of force passing by the point it came from. In this manner the fact that the points of insertion are always attached at the end of a fiber would be intelligible. The interpretation given in the present paper, however, seems to be more adequate.

Steatococcus case. In spindle structure and anaphase chromosome movements the Mexican coccid *Steatococcus tuberculatus* recently studied by Hughes-Schrader and Ris (1941) presents a striking similarity to *Tityus bahiensis*. Thus, in metaphase the chromosomes of Steatococcus lie with their entire body on the plane of the equator; at anaphase the chromosomes separate in per-

fect parallelism showing besides the fibers attached at their extremities many others distributed along the whole length of their polar face; in the end of anaphase the chromosome extremities bend toward the poles.

Steatococcus chromosomes, however, differ from Tityus chromosomes in the complete absence of the end repulsion, in consequence of which they maintain their metaphase parallelism until the end of anaphase, when they bend their ends toward the corresponding pole. The absence of any localized repulsion making impossible the detection of the position of the kinetochores, led the authors to the assumption of diffuse attachment, that is, of individual half spindles extending from one extremity to the other of the polar side of the chromosomes. Nevertheless, there is a particularity in the behavior of the Steatococcus chromosomes which permits a quite different interpretation. I mean the bending of the extremities of the chromosomes toward the poles in the end of anaphase. The authors did not pay any special attention to this fact, considering it as the effect of pressure on the whole chromosome from the narrowing space of the polar cone.

In the opinion of the present writer, the chromosomes of Steatococcus are, like Tityus chromosomes, provided with a point of attachment at each extremity. But, being relatively thicker and probably more rigid than in Tityus, the chromosomes of Steatococcus oppose resistance against the bending effect concentrated in both extremities, which, in this manner, can not be distinguished from the more or less generalized effect tending to separate the halves of the chromosomes. The resistance of Steatococcus chromosomes against bending is increased by the fact that each chromosome is constituted by two parallel chromatids. In the end of anaphase, however, due to a decrease in the viscosity of the chromosomes they become concave toward the corresponding pole, thus revealing the greater kinetic power of the extremities.

The explanation of Hughes-Schrader and Ris for the bending of the chromosomes seems to me inconsistent because if we admit the presence of a diffuse kinetochore, then we are forced to assume that the chromosomes, compelled to become bent when they reach the narrower parts of the spindle, would do so exactly in an opposite direction, that is, with the convexity facing the poles. If they really penetrate the polar zones of the spindle with the ends directed forwardly it is because the kinetic centers are localized at the ends.

As was referred to above, chromosome fragments of Steatococcus obtained by means of x-ray treatment behaving themselves like unbroken chromosomes were taken as proof for the reality of the diffuse kinetochore. This question will be discussed below.

Protenor case. The case of the X-chromsome of Protenor and of all other long chromosomes which behave in the same way may be joined to Tityus and Steatococcus cases. What makes the chromosomes of Tityus and Steatococcus take in the equatorial plane such a position that the components of each pair or double element look toward opposite poles is the existence of two kinetochores (undivided as in Tityus or already divided as in Steatococcus) at their ends. Mitosis studied in the embryos of Tityus as well as of Steatococcus has shown that in metaphase the separation of the chromosomes is already complete. There being two kinetochores directed toward opposite poles in each extremity of the double metaphase chromosomes, they separate parallel to each other.

The X-chromosome of Protenor, in the first division of the spermatocytes, which is equational, is evidently in an identical situation. The fibers attached along the entire length of the polar face of the X-chromosomes and their parallelism at anaphase led Schrader (1935) to propose for the first time a diffuse mode of spindle attachment. However, the behavior of this chromosome passing undivided to one of the poles in the second mitosis was left without explanation, since it moves parallel to the

spindle axis and maintains the extremities connected with the corresponding pole by means of a delicate fiber. But it seems to me that this peculiarity may be accounted for by the presence of a kinetochore at each end of the chromosome. Really, the kinetochores being single and localized just at the ends as Schrader's figures suggest, the influence the poles exert on them sooner or later would force the chromosome to take a position more or less parallel to the axis of the spindle.

A New Theory of Meiosis

Taking all these facts into consideration I will try in the following paragraphs to develop a general theory of meiosis based on the dorsoventrality of the chromosomes established in them by the activity of the kinetochore, as was already outlined in a previous paper (Piza, 1942).

The kinetochore is an universal morphological particularity of the chromosomes as important for them as the nucleus for the cell (Piza, 1941a). Without a kinetochore chromosomes can not exist. Besides any other function which may be ascribed to it, the kinetochore must be considered as the kinetic center of the chromosomes.

Chromosomes attract and repel one another as wholes (cytologically demonstrated by Piza, 1942). Repulsion seems to be a permanent property of chromosomes (Darlington, 1937; Koller, 1934; White, 1937), while attraction is to be regarded as a new property confined to homologous chromosomes periodically conferred on them by the kinetochore. At prophase of meiosis the attraction power developed between homologous chromosomes overcoming the repulsion power, they approach one another. The attraction power does not suppress the opposite one which continues to prevail among non-homologous chromosomes. In the beginning, the activity of the kinetochore seems to confer on the whole chromosome a weak and more or less generalized power of attraction, so that pairing begins by chance at any part of the chromosomes. Later, the attraction power without disappearing

from the rest of the chromosomes becomes concentrated in the kinetochores, thus promoting their coincidence. Due to this primary coincidence all other parts of the chromosomes sooner or later finish by coinciding too. In the course of the development of the chromosomes the kinetochore becomes localized at one of their sides, establishing in this way a clear dorso-ventrality in the chromosomes, cytologically demonstrable (Piza, 1942). Hereafter chromosomes attract one another by the ventral (kinetochore) side. The attraction power is stronger at the kinetochore than at any other part of the ventral side of the chromosomes. The formerly generalized repulsion power, which never disappears from the chromosome body, is now displaced to the dorsal side, remaining stronger at the kinetochore region. In chromosomes like those of Tityus provided with a kinetochore at each end the attraction power seems to be effective along the entire ventral side, in consequence of which the paired elements can untwist till they become perfectly parallel. In metaphase, chromosomes are united venter by venter. The poles attract the ventral side of the chromosomes and repel the dorsal one. At metaphase, the ventral side of the chromosomes being unexposed to the polar attraction, the poles, acting on their dorsal side, repel them to the plane of the equator. In anaphase the disorganization of the chromosomes, sometimes very pronounced at the end of this phase, begins by a decrease in the activity of the kinetochore and consequently of the ventral side, so that, when the mutual attraction power of this face becomes smaller than the attraction power the poles exert on it, the chromosomes rotate. Then, offering the ventral side to the polar attraction and facing one another with the repelling dorsal sides, the chromosomes separate and anaphase proceeds.

In chromosomes provided as ordinarily with a single kinetochore the kinetic activity of the ventral side may be more or less concentrated in it, so that separation begins at this point.

Secondary spindle fibers, that is, fibers without direct connection with the kinetochore, may be formed between the poles and a more or less extensive area of the ventral side of the chromosome, in accordance with the activity conferred to this side by the kinetochore. In Tityus, Steatococcus and in the X-chromosome of Protenor the whole ventral side shows kinetic activity and determines spindle fiber formation. Most chromosomes with a single kinetochore are attached at the extremity of a bundle of fibers, parallel or spread out like the sticks of a very delicate fan, revealing in this way a certain kinetic activity in the neighborhood of the kinetochore.

The hypothesis that each chromosome is dorsoventrally differentiated is not new. The first investigator to propose such an interpretation was Cooper (1938), who suggested that "the synapsing chromosomes are bilateral in organization, *i.e.*, constructed in such a manner that each chromosome possesses but one, limited, pairing surface."

Schrader (1940) approached very closely to the present interpretation of mitosis when he wrote: "So far as I can see, there are just two ways of escape from the difficulty: one, that the negative charge of either chromosome or center changes to a positive one or else becomes weak or neutral, while the movement is going on; the other, that, unlike the main mass of the chromosome, the kinetochore is positive and thus is not repelled by the center."

Pease's experiments, demonstrating that the rate of chromosome movement in anaphase decreases with the fluidification of the spindle, do not contradict the idea of an attraction exerted by the poles, considering that no modifications introduced by hydrostatic pressure, either into the poles or into the chromosomes and capable of altering the movement of the latter, were taken into account. Likewise, Shimamura's investigation on the effects of centrifugal force on nuclear division (1940) do not contradict the conclusions of the present paper with regard to the uselessness of the spindle fibers in attaching

the chromosomes to the poles or in moving the chromosomes, since what the experiments have really brought into evidence was merely the great power of the force which maintains the kinetochore turned toward the poles even when the body of the chromosome has been projected to the centrifugal side. The effect of centrifugation on material previously submitted to chloralhydrate and colchicine treatment has demonstrated in its turn that in the treated cells, if the poles did not suffer any modification in their attraction power (?), the chromosomes at least have lost the faculty of reacting to the influence of the poles, becoming incapable of moving and consequently of producing spindle fibers.

The Behavior of the Chromosome Fragments of Steatococcus

This question will become more comprehensible when we get a more extensive information about the structure and function of the kinetochore. For the moment we can only consider the kinetochore not yet marked by the presence of a granule as representing a more primitive state in the story of this element. In this case we can assume that the kinetochore region of the chromosome may be represented in the beginning of prophase by a more or less extended area which would be more and more restricted as the nucleus proceeds in its mitotic differentiation. Every chromosome fragment of Steatococcus which behaves like an unbroken chromosome must have at least a kinetochore originated from fragmentation of the kinetochores of the normal chromosomes, which must have occurred at a time when the chromosomes and most probably the kinetochores still were in a state of great distention. This assumption is in full agreement with Schrader's (1939) suggestion "that there is some reorganization of the kinetochore at every mitotic cycle and that functional fragments, such as reported by McClintock, originate at a time when the final form shown in metaphase has not yet been assumed."

Chromosome fragments associated with a whole kinetochore or with a large piece, would behave like normal chromosomes, their parallelism at anaphase being accountable for by an uniform distribution of the kinetic activity along their entire ventral face. The chromosome fragments provided with a rather small piece of the kinetochore, due to the insufficiency of it, would lag and finally would be lost. Finally, fragments of chromosomes entirely unprovided with kinetochore, might show some kinetic activities due merely to the normal repulsion power of the chromosomes still present in them, but sooner or later would vesiculate and disappear.

Summary

The meiotic chromosomes of *Tityus bahiensis*, provided with a kinetochore at each extremity, are attached at the spindle long before metaphase is reached. Being still twisted and bent at that time, they have to perform many varied movements in order to assume their rod-shaped metaphase form. Since the spindle fibers are not altered in any way by the movements of the chromosomes it was concluded that they are nothing else than the inevitable consequence of a very feeble coagulation process developed continuously between the kinetochores and the pole, following exactly the line connecting these points and without any influence in moving the chromosomes.

Steatococcus chromosomes and the X-chromosome of Protenor, like Tityus chromosomes, are considered as being provided with two terminal kinetochores. An explanation based on the dorso-ventrality of the chromosomes is proposed for the presence of fibers along the entire polar face of the chromosomes.

The presence of a kinetochore at each end makes intelligible the fact that the X-chromosome of Protenor, in the second meiotic division of the spermatocytes, moves parallel to the spindle axis toward one of the poles.

A new theory of meiosis, based on the dorso-ventrality of the chromosomes, is presented.

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REVIEWS AND COMMENTS

EDITED BY CARL L. HUBBS

In this section reviews and notices are given of current publications on general biology and of specialized works which have an important bearing in this general field. Emphasis is given to books and major articles which fall within the special scope of The American Naturalist, in that they deal with the factors of organic evolution.

Reviews and Comments are meant to include also such general discussions, reports, news items and announcements as may be of wide interest to students of evolution. Except as otherwise indicated, all items are prepared by the Section Editor, Dr. Carl L. Hubbs, University of Michigan, Ann Arbor, Michigan. All opinions are those of the reviewer.

The Genetics of the Mouse. By Hans Grüneberg. Cambridge: At the University Press; New York: The Macmillan Company, 1943: i–xii, 1–412, pls. 1–14, figs. 1–43. $7.00.

This comprehensive and authoritative survey of the genetics of the house-mouse (*Mus musculus* and *M. bactrianus*) will be of great value to all those numerous investigators who utilize this common animal in their laboratories. It will be of particular interest to geneticists, anatomists, physiologists and pathologists. More is known about the heredity of the house-mouse than about that of any other laboratory mammal. The number of inherited variations that have been discovered in this rodent is very considerable, but because of the uncertain status of many characters the exact number of known genes cannot be stated.

Practically every part of the body of the house-mouse exhibits hereditary modifications of one sort or another. The total body size may be decreased or increased; the tail, limbs, or external ears may be reduced in size or variously deformed; the eyes may be rudimentary or absent; and there may be cleft lip and palate. There are many striking modifications of the pelage leading to the production of numerous variations of color, including various types of spotting with white; the hair may be variously curled or misshapen, or may be absent during a part of the life-history, leaving the animal naked. The bones may be greatly modified, especially in the head, limbs, and tail; in one strain there is congenital absence

of the tibia. Inherited abnormalities have been described for the pituitary, thyroid and adrenal glands, for the urinogenital system, for the blood and blood-forming organs, and for other internal organs. There are inherited differences in resistance to certain types of disease, including cancer, and also differences in serological reactions. The brain and organs of special sense also exhibit a number of inherited abnormalities and certain types of blindness and deafness are thereby produced. There are inherited differences in behavior, in learning ability, in wildness and savageness, and in social behavior, but the precise genes involved in most of these psychological differences have not yet been analysed.

A brief but valuable summary on "The Genetics of Cancer in Mice" is contributed in an appendix by C. C. LITTLE and P. A. GORER.

"These structural variations and pathological processes show a considerable likeness to human affections, and the day will come when human pathologists will realize the value of the inherited diseases of the mouse in solving problems of human pathology, where observation is often difficult and experimentation usually impossible. Hitherto, the majority of human pathologists are hardly aware of this promising material . . ." Investigations in anatomy, embryology, physiology, pathology and psychology would often be benefited by the use of an animal such as the house-mouse, whose genetics have been at least in part worked out. The similarities of the house-mouse to man in many of its characters make it particularly appropriate for investigations in these fields. The genetics of other kinds of laboratory mammals have been worked out less thoroughly than that of the house-mouse. The author fails to point out, however, that some of the other laboratory animals would, because of their larger size, be more suitable than the mouse for certain types of studies.

It is surprising that inherited epilepsy has not yet been described for the house-mouse, although inherited types

of other behavior defects, such as waltzing, shaking, and circling have long been known. A number of inherited types of abnormal behavior, sometimes including convulsive seizures, are known for *Peromyscus* and for the house-rat and it is the belief of the reviewer that careful investigation will discover similar types of hereditary convulsive behavior in the house-mouse.

Among some of the more interesting genes are those that affect several different parts of the body. For instance, the gene for siderocyte anemia often produces also a flexed tail and a white spot on the belly. A number of the other genes are known to produce at least two entirely different sorts of effects. Although geneticists are aware that every gene probably affects several different organs or indirectly the whole organism, it is not always easy to demonstrate that such widely divergent effects actually are produced by the same gene.

A group of genes of particular interest to geneticists are those that change in dominance depending on their genetic background. The piebald gene *W*, for instance, may be either dominant, semidominant or recessive, depending upon the modifiers with which it is associated.

Phenotypic effects that appear to be similar may be and frequently are produced through the action of entirely different genes. Warning is therefore given against attempts to homologize genetic characters in different animals on the basis of the phenotypic effects alone. In the opinion of the reviewer, this warning is particularly pertinent in the field of human genetics. It is never safe to assume that a similarly appearing character that occurs in two independent human kindreds actually is due in both cases to the same gene. Much controversy about the mode of inheritance of human traits can be avoided if this warning is kept in mind.

The number of chromosomes in the house-mouse is forty, of which one pair form an XY combination related to sex. There are twenty possible linkage groups. One of the genes concerned in the transplantability of a can-

cerous tumor is known to be sex-linked, but no gene producing a visible effect has been demonstrated to be located on the X-chromosome. By linkage tests eight of the autosomes have been demonstrated each to carry at least two genes. Only one mouse chromosome, however, has been definitely proved to be the carrier of as many as three genes.

A comprehensive bibliography of 1,141 titles is given. This is arranged according to subject, but the contributions of individual authors may be found through the author index.

The publication of such a book as this in war-time England is a notable achievement. Nevertheless, the price of $7.00 for a book of this size is greatly in excess of that usually asked for books of the same character printed in the United States. The high price is to be regretted, for this will prevent the purchase of the work by many of the persons who would profit by its possession.

LEE R. DICE

Joseph Grinnell's Philosophy of Nature Selected Writings of a Western Naturalist. Berkeley and Los Angeles: University of of California Press, 1943: i–xv, 1–237, 13 figs. and pls. $2.00.

A SPLENDID idea, a worthy tribute to a great naturalist, a significant contribution to the philosophy of evolution!

To the end of his days Joseph Grinnell kept too busy with special researches to yield to requests that he write books which would bring together and make generally available his highly respected views on the relations between organisms and their environment. He had, however, always been a penetrating and clear thinker as well as an exceptionally productive researcher, and he had scattered through his special reports wise generalizations which had developed from these researches and thoughts. Alden H. Miller and other devoted students and colleagues found it possible to supply the long-felt need, by selecting from Grinnell's large bibliography a series of papers, and sections of others, that reflect the views of this master

naturalist on general systematics, ecology, zoogeography, conservation and speciation.

"Joseph Grinnell's Philosophy of Nature" takes the form of a series of essays, delightfully written and chronologically arranged; interesting enough to induce the reader to complete the book, yet sufficiently short and independent to permit the separate reading of the items, during the fragments of time that these busy days leave us for general reading. Though the extracts are of works published during one third of a century (1903 to 1936) the views expressed are by no means outmoded. Grinnell consistently thought ahead of his time. It is a source of surprise to note the date of original publication of viewpoints that would reflect the keenest modern thought on evolutionary problems.

Grinnell approached the problem of evolution through the analysis of the environmental relations and the characters of subspecies—species caught in the act of evolution. He held that each form evolves by natural selection, becoming thoroughly adapted to a particular ecological niche, and that its differentiation is rigidly conditioned by isolation. The following points are particularly stressed:

> The course of organic evolution has been molded and is being molded by environmental circumstance. In one sense this is directed evolution—orthogenesis of a kind. . . . Plant-animal communities . . . have been subject to evolutionary processes quite as definitely as discrete species.

Chimpanzees A Laboratory Colony. By ROBERT T. YERKES. New Haven: Yale University Press, 1943: i–xv, 1–321, frontisp. + pls. 1–63, figs. 1–24. $5.00.

THE story of the chimpanzee is entrancingly told, but so comprehensively that the recitation of experiments, observations and anecdotes has been cut to tantalizing briefness. After finishing Yerkes' latest book one will be sorely tempted to study other books and articles on the great apes, and if blocked in this desire by the high premium which these overcrowded days place on general reading, he may hold toward the author feelings other

than those of unmitigated gratitude. If so, the times are to be blamed.

Yerkes treats chimpanzees as animals preadapted to the solution of problems by preceptual, presumably ideational, responses, at time "by a clear vision of the solution." In their minds as well as in their bodies and their diseases he looks on these apes as almost human. In their ways of life and in their mental reactions he sees, at least as a glimmer, the basis of the human essentials—socialization, symbolism, language and culture. Yet he has obviously striven to understand the chimpanzee mind as a distinctive entity. He stresses in particular the importance of the positional arrangement or relative direction of objects in the memory and delayed responses of this ape. From his psychobiological viewpoint Yerkes presumably joins most modern zoologists and anthropologists in regarding the chimpanzee as our cousin rather than as our ancestor.

The book is loaded with interest for the zoologist as well as the psychologist, the pathologist, the animal keeper and the sociologist. It contains a wealth of sound bilological philosophy. Thus, in treating the social development of the individual, he is convinced "that heredity and environment are inseparables, whose importance varies for different patterns of response and phases of organic development."

Outstanding among many treats is the autobiographical account of the early conception, the long promotion, the final consummation and the full justification of the Yale Laboratories of Primate Biology, recently placed on a continuing basis and very appropriately renamed the Yerkes Laboratories. Yerkes promises science, in the final statement, that his release from the administration of this already amazingly productive institute "is not the end, for essentially preparatory work now can give place largely to fundamental research." May he be given many years to further his own analyses of primate culture, and to aid him in his expressed desire of contributing to the foundation of human engineering!

Speaking of Man A Biologist Looks at Man. By MICHAEL F. GUYER. New York and London: Harper and Brothers, 1942: 1–321. $3.50.

CONGRATULATIONS are due to Professor Guyer, for his help in dispelling the idea that noted research biologists are encloistered recluses who have lost their ability to associate and converse with the common man. He speaks well and understandingly. Some passages, indeed, are superb combinations of deep, clear thinking with satire and wit. Had such style been sustained, the author's reputation would no doubt have passed beyond that of a master of popular scientific style to that of a literary genius.

What Dr. Guyer speaks about so well is not merely, in fact in rather small part, the biology of man. His ten talks cover a broad range of subjects: Biology and the Happy Life, Science and Its Critics, Man's Place in Nature, The Rise of Intelligent Behavior, Managing Our Minds, The Endocrine Control of the Body, Sex, Democracy as a Biological Problem, The Educated Failure, Man's Search for the Ideal. These essays constitute a frank philosophy of human life, that should lead many to a trurer and more wholesome outlook.

NOTICES OF NEW BOOKS

General Zoology. By TRACY I. STORER. New York and London: McGraw Hill Book Co., 1943: i–xii, 1–798, 5 col. pls., 551 figs. $3.75. This is a marvelously informative text and general reference book on zoology. Perhaps its most impressive features are the many new illustrations, which strikingly clarify the text discussions and must surely incite the interest of any worthwhile student. The eye is particularly arrested by the colored plates which boldly exhibit the internal anatomy as semi-transparencies and by the ecological diagrams which illustrate differential habitat selection. The whole treatment, first under topical and then under systematic arrangement, is remarkably thorough and well balanced. Many zoologists of the future will be better zoologists for having received their inspiration and early training from Tracy Storer's book.

Animal Breeding Plans. Second Edition. By JAY L. LUSH. Ames: Iowa State College Press, 1943: i–viii, 1–437, figs. 1–50. $3.50. Lush's comprehensive and authoritative advanced textbook on animal breeding passes into its second edition. Changes are greatest in the chapters on Genetic Basis of Variation and on Family Structure of Populations. The theoretical as well as the applied aspects of animal breeding are dealt with, and particular stress is laid on the problems involved in the improvement of livestock. The book is of great interest and value, however, to the general zoologist, the geneticist and the student of speciation. This is particularly true of the analyses of the nature of differences between breeds, the operation of selection, the structure of populations, inbreeding, line breeding and outbreeding—in all of which the statistical genius of Sewall Wright has been heavily relied upon.

AMERICAN SOCIETY OF NATURALISTS

As a result of its annual balloting the American Society of Naturalists has elected the following officers to serve for the year 1943: H. J. Muller, Amherst College, *President;* B. M. Duggar, University of Wisconsin, *Vice-President;* A. C. Kinsey, Indiana University, *Secretary;* M. R. Irwin, University of Wisconsin, *Treasurer.*

The following persons were elected, on account of outstanding achievement in biological research, as members of the society: Ernest C. Abbe, LeRoy Abrams, F. A. Beach, J. P. Bennett, James Bonner, Ralph Buchsbaum, Earl O. Butcher, Fred K. Butters, Wanda K. Farr, David R. Goddard, Karl C. Hamner, Edwin R. Helwig, Hope Hibbard, Theodore L. Jahn, John S. Karling, Stewart A. Koser, Alfred M. Lucas, Gordon Marsh, H. M. Parshley, Frederick V. Rand, P. L. Risley, Ralph Singleton, J. M. Webber, S. H. Yarnell.

SHORTER ARTICLES AND DISCUSSION

INHERITANCE OF MOTTLED EARLOBES AND STUBS IN RHODE ISLAND REDS[1]

In recent years the appearance of mottled earlobes in Rhode Island Reds bred for high fecundity has been rather widespread. The amount of white mottling varies widely between individuals from a few whitish areas to large areas in which the earlobe is essentially white.

Warren (1928) observed that many of the so-called red earlobe breeds do not breed true for red earlobes. In his study of crosses between Rhode Island Reds and Leghorns there was evidence of several factors operating to modify earlobe color, probably two autosomal genes, but there was no evidence of sex-linked genes affecting the amount of white in the earlobes of Rhode Island Reds.

The appearance of mottling in the earlobes of Rhode Island Reds is objectionable according to breed standards, and breeders have had considerable difficulty in eliminating it from their flocks. Just why this variation should become more prevalent during the last decade is not clear. The possibility exists, however, that in breeding for high fecundity there have been more related matings which would lead to genetic segregation.

Stubs are rather common in many breeds that normally lack any evidence of feathers on shanks or toes. The appearance of down between the toes or on the shanks of Rhode Island Red chicks at hatching is not uncommon, but this down does not usually persist longer than a few days. There is no evidence in our flock that down on the shanks or toes is a precursor of stubs at maturity. The appearance of down on the toes and shanks that was observed by Warren (1930) in White Leghorn adults was not observed in our Rhode Island Reds. Stubs in our stock consisted of short feather quills on the shanks or between the toes.

Lambert and Knox (1929) present a review of the literature on shank feathering which need not be repeated here. They are inclined to agree with Serebrovsky (1926) that probably four genes are concerned in shank feathering and that there is evidence that recessive genes may be responsible for stubs in clean shanked breeds.

[1] Contribution No. 464 from the Massachusetts Agricultural Experiment Station.

EXPERIMENTAL DATA

This study was undertaken to discover the mode of inheritance of mottled earlobes and stubs within the Rhode Island Red breed. The investigation began with the generation hatched in 1935 and carried through eight generations concluding in 1942. During this period 7 different sires were mated to 58 dams to produce 820 offspring that reached the age of six months. Records were made at hatching for down on the shanks or toes and at the approximate age of six months for stubs and earlobe color in the complete families.

Each generation was sired by a single male mated to several hens. The second and third generations were sired by two brothers from the first generation so that there was some inbreeding. An outside male with red earlobes and stubs was used to produce the fourth and fifth generations. The last three generations were each produced by a son of the preceding generation so that inbreeding was practiced. The sires used in 1940 and 1941 had mottled earlobes and stubs and the sire used in 1942 had red earlobes and clear shanks.

INHERITANCE OF MOTTLED EARLOBES

In this experiment some inbreeding was practiced in that sons were often mated back to their dam and were also mated to their half sisters and one sire that was used for two years was mated to several of his daughters. A careful study was made of the phenotypes produced from mating normal sires to normal dams, normal sires to mottled dams, mottled sires to normal dams and mottled sires to mottled dams.

There was good evidence that inbreeding increased the incidence of mottling, but there was evidence that the mottled condition may not always appear at the age of six months and may be observed in the same individuals in the spring of the second year when the birds are about twelve months old.

Common phenotypic ratios observed were: 9 to 7 and 3 to 1 when normal phenotypes were mated. When one parent was normal and the other mottled the common ratios were: 1 to 1, 3 to 5 and 1 to 3. When both parents showed the mottled condition the usual phenotypic ratio of the offspring was 1 to 1. The fact that matings where one parent was normal and the other mottled often gave 1 normal to 3 mottled indicates that more than one recessive gene is concerned. When mottled is mated

to mottled the phenotypic ratio of the offspring is often 1 to 1. This is evidence that either of two recessive genes may produce mottling. A single recessive gene would not give phenotypical ratios like those observed and there was no evidence of sex-limited or sex-linked inheritance.

In Table 1 the data are summarized by phenotypes for the different matings to save space. The most probable genotypes of the parents are indicated.

TABLE 1

COMBINED RESULTS OF MATING FOR MOTTLED AND NORMAL EARLOBE COLOR, 1935–1942

	Sons		Daughters			Totals	
5 Normal Sires ($R_1r_1R_2r_2$) × 19 Normal Dams ($R_1r_1R_2r_2$)	Normal	Mottled	Normal	Mottled		Normal	Mottled
	136	67	100	94		236	161
					Expect	223	174
5 Normal Sires ($R_1r_1R_2r_2$) × 22 Mottled Dams ($R_1r_1r_2r_2$)	Normal	Mottled	Normal	Mottled		Normal	Mottled
	67	89	45	107		112	196
					Expect	115	193
2 Mottled Sires ($R_1r_1r_2r_2$) × 8 Normal Dams ($R_1r_1R_2r_2$)	Normal	Mottled	Normal	Mottled		Normal	Mottled
	17	20	34	18		51	38
					Expect	33	56
1 Mottled Sire ($R_1R_1r_2r_2$) × 4 Mottled Dams ($r_1r_1R_2r_2$)	Normal	Mottled	Normal	Mottled		Normal	Mottled
	8	6	7	5		15	11
					Expect	13	13

From the matings of birds with normal red earlobes there were produced 236 normal to 161 mottled offspring or a 9 to 7 ratio. This would indicate that two recessive genes r_1 and r_2 must be present in the stock. Each of these recessive genes must be covered up by its allele to give the normal red condition.

When normal males were mated to mottled females the offspring showed 112 normal to 196 mottled. This ratio would be approached if the sires were heterozygous for both genes and the mottled dams were pure for one recessive gene and heterozygous for the other.

The reciprocal cross mottled males on normal females gave too few mottled female offspring but a reasonable approach to expectation.

A single mottled male mated to four mottled females gave 15 normals to 11 mottled while equality was expected. This mating gives further evidence that either of two recessive genes may produce mottling. The fact that normal × normal gives both

normal and mottled indicates the dominant nature of genes for red earlobe. There is no evidence in our data of sex-linked genes being concerned in the mottled earlobe condition.

INHERITANCE OF STUBS

The stock of Rhode Island Reds used in the study of mottled earlobes was also classified with respect to the presence or absence of stubs in complete families at six months of age. This study began in 1936 and includes seven generations.

Stubs appear to behave in inheritance in identical fashion with the mottled earlobe condition, although the two characters are entirely independent. A careful study was made of phenotypic ratios from different types of matings through a seven year period. It was noted that stubs can be recognized by careful examination at six months of age. They show a tendency to disappear in some birds during the first laying year. In this study all records were made at six months in both males and females.

In Table 2 all data are grouped according to phenotype and parents.

TABLE 2

COMBINED RESULTS IN MATINGS FOR CLEAR SHANKS AND STUBS—1936–1942

	Sons		Daughters		Totals	
3 Clear-Shanked Sires × 24 Clear-Shanked Dams	Clear	Stubs	Clear	Stubs	Clear	Stubs
	162	20	150	16	312	36
1 Clear-Shanked Sire ($S_2S_2S_4S_4$) × 3 Dams with Stubs ($s_2s_2S_4S_4$)						
	2	2	4	1	6	3
				Expected	7 to	2
3 Sires with Stubs ($s_2s_2S_4S_4$) × 18 Clear-Shanked Dams ($S_2S_2S_4S_4$)						
	86	40	110	34	196	74
				Expected	203 to	67
2 Sires with Stubs ($S_3S_3s_4s_4$) × 4 Dams with Stubs ($s_2s_3S_4s_4$)						
	12	12	9	8	21	20
				Expect	20.5 to	20.5

Stubs in Rhode Island Reds appear from the above data to be produced by either of two recessive genes s_3 and s_4 that are not sex-linked. For example, the mating of 1 clear-shanked sire to 3 dams with stubs gave 6 with clear shanks to 3 with stubs which closely agrees with expectation. Three sires with stubs when mated with 18 clear-shanked dams gave 196 clear to 74 with

stubs while the expectation was 203 to 67. Mating two sires with stubs to 4 females with stubs gave 21 clear to 20 with stubs when equality was expected. A deficiency of females showing stubs occurs in our data, as was observed by Lambert and Knox (1929). It is our belief that this character is partially sex-limited.

Discussion

Within the Rhode Island Red breed evidence indicates that the mottled earlobe condition is produced by two recessive autosomal genes designated as r_1 and r_2. No evidence is available on possible cumulative effects of these genes but the extent of mottling varies rather widely. Because of the nature of inheritance it is very difficult to entirely eliminate the mottled earlobe condition from Rhode Island Reds.

Apparently stubs are inherited in a similar fashion to the mottled earlobe condition. Two independent recessive autosomal genes are indicated, genes s_3 and s_4. Cumulative effects on the extent of leg feathering produced by these genes have not been determined. This undesirable character is also very difficult to eliminate from the flock.

For purposes of completely eliminating either mottled earlobes or stubs in Rhode Island Reds, an inbreeding program offers possibilities of uncovering these recessive genes. There was no evidence of any linkage between any of the four genes.

Conclusions

Mottled earlobes in Rhode Island Reds appear to be produced by two recessive autosomal genes.

Stubs in Rhode Island Reds seem to depend upon two recessive autosomal genes.

No evidence of linkage was observed between genes for mottled earlobes and genes for stubs.

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RELATIONSHIP BETWEEN THE LENGTH AND THE WEIGHT IN THE SNAPPING TURTLE *CHELYDRA SERPENTINA* LINNAEUS[1]

IT is now well established for fishes that weight (W) usually may be expressed as a function of length (L) by means of the equation, $W = C L^n$, where C and n are empirically determined constants. To our knowledge, however, such a relationship has not heretofore been shown for any chelonion species. Data are here presented on the length and weight of snapping turtles (*Chelydra serpentina*) and are analyzed to show the nature of the relationship between length and weight for this species and to provide a practical means for approximating weight when length is known and *vice versa*.

The measurements that we have used were made on 151 common snappers from the Lower Peninsula of Michigan. These specimens were collected in lakes, ponds and streams during the summers of 1937 and 1938, mostly by means of traps. All were weighed alive, at the time of capture, on a spring balance accurate to the nearest $\frac{1}{4}$-pound. The length, also obtained at time of capture, is the greatest over-all length of the carapace as measured horizontally through it by means of a large caliper, accurate to the nearest $\frac{1}{8}$-inch.

Preliminary analyses failed to reveal sexual dimorphism for the features studied and for our purposes sex is disregarded. Males numbered 74, females, 77.

The equation, $W = C L^n$, reduced to logarithmic form, may be written: $\log W = \log C + n \log L$. The equation may be applied to observational data, therefore, by fitting a straight line to the logarithms of the empirical lengths and weights. In the present study the equation was not fitted to the data for the individual turtles but rather to the average lengths (in millimeters) and weights (in grams) of turtles within half-inch intervals of length.

The value of C may be computed from the formula:

$$\text{Log } C = \frac{[\Sigma \log W \cdot \Sigma (\log L)^2] - [\Sigma \log L \cdot \Sigma (\log L \cdot \log W)]}{[N \cdot \Sigma (\log L)^2] - (\Sigma \log L)^2} \qquad (1)$$

[1] Contribution from the Department of Zoology, University of Michigan, and the Institute for Fisheries Research of the Michigan Department of Conservation. Financial aid was given the field work on which this paper is based by the Associated Fishing Tackle Manufacturers and by the American Wildlife Institute.

Where N = the number of points (15) in the empirical data. The value for the logarithm of the constant was found to be – 3.789234.

The value of n was found to be 3.06383, as determined by the application of the formula,

$$n = \frac{\Sigma \log W - N \cdot \log C}{\Sigma \log L} \qquad (2)$$

The length–weight equation for snapping turtles from the Lower Peninsula of Michigan is therefore:

$$\log W = -3.789234 + 3.064 \log L \qquad (3)$$

or

$$W = 1.625 \times 10^{-4} L\ 3.064 \qquad (4)$$

Since the value of n is very near to 3, the length–weight relationship of this turtle would appear to approximate the "cube law" fairly closely. With a few exceptions, the n in equations fitted to the length–weight data of fishes has not deviated far from 3, and values above that figure have been more numerous than exponents less than 3.

Comparisons between empirical weights and weights calculated by means of equation (3) may be had from the data of Table 1

TABLE 1

LENGTHS AND WEIGHTS (AVERAGES FOR HALF-INCH SIZE GROUPS) OF SNAPPING TURTLES AT CAPTURE AND THEORETICAL WEIGHTS AT THE VARIOUS LENGTHS AS COMPUTED FROM THE LENGTH–WEIGHT EQUATION

Number of Turtles	Length (Inches)	Weight (pounds)		Length (Millimeters)	Weight (grams)	
		Actual	Calculated		Actual	Calculated
2	7.75	4.3	3.8	197	1,873	1,740
4	8.25	5.3	4.7	210	2,404	2,116
16	8.75	5.8	5.5	222	2,631	2,509
20	9.25	6.7	6.6	235	3,039	2,987
25	9.75	7.4	7.8	248	3,357	3,523
25	10.25	8.7	9.0	260	3,946	4,071
16	10.75	9.6	10.4	273	4,355	4,728
9	11.25	11.3	11.9	286	5,126	5,420
12	11.75	13.3	13.6	298	6,033	6,184
1	12.25	15.5	15.4	311	7,031	6,999
8	12.75	16.9	17.6	324	7,666	7,990
10	13.25	18.7	19.9	337	8,482	9,014
2	13.75	22.0	22.1	349	9,979	10,034
1	14.25	25.0	24.7	362	11,340	11,220
1	14.75	30.0	27.6	375	13,608	12,505

and Fig. 1. In general, the theoretical length–weight curve fitted the empirical data rather well. Fairly large discrepancies occurred at some intervals, it is true, but these irregularities can be explained satisfactorily as the result of the small number of turtles in the collection. Six of the 15 half-inch intervals were represented by less than five turtles each, and three of these six were represented by only one specimen.

It is concluded, therefore, that the length–weight relationship of snappers from the Lower Peninsula of Michigan over the

length range of 7.5 to 15 inches may be expressed satisfactorily by the general equations (3) and (4). It is possible also that the extrapolation of the curve for a reasonable distance outside this length range may give satisfactorily accurate results. The equation and graph therefore provide useful methods of estimating weight when length alone is known.

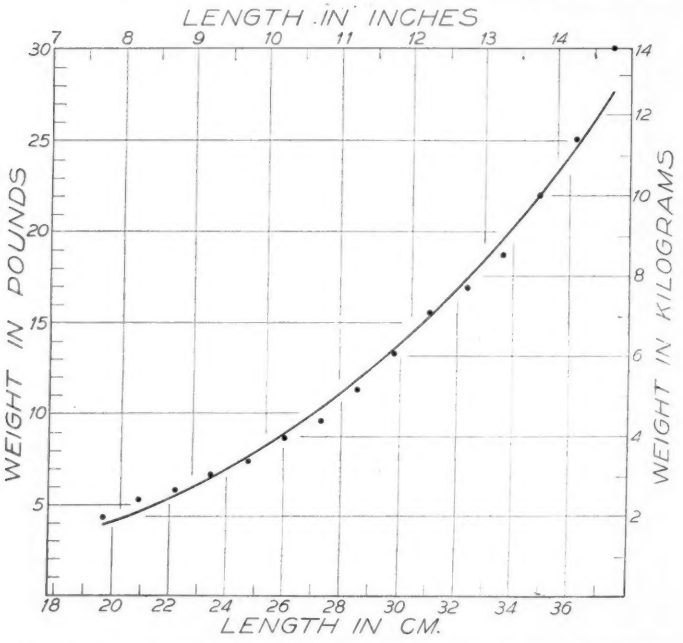

FIG. 1. Length–weight relationship of the snapping turtle in the Lower Peninsula of Michigan (sexes combined). The curve is the graph of the equation fitted to the length–weight data and the dots represent the empirical averages of length and weight.

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PARTIAL OOSORPTION AS A POSSIBLE CAUSE OF DIPLOID MALES IN MICROBRACON HEBETOR

THE occurrence of sex reversal in newly hatched larvae of *Tribolium* by means of starvation (Holdaway, 1930) and in the larvae of certain Hymenoptera by means of parasitization (Wigglesworth, 1940) indicates that the extraction of nutriment from ripe ovarian eggs subjected to the process of oosorption may have a similar effect if such eggs are fertilized and deposited before their viability is destroyed.

In the Hymenoptera, biparental males are known to occur only in *Microbracon hebetor* (Whiting, 1935). Such males are not sex-reversed females, according to inheritance studies made by Whiting (1935), if the occurrence of biparental males is the result of some condition correlated with the interbreeding or close relations of the parasites. However, it was observed by Whiting that in *M. hebetor* there is a definite correlation between the occurrence of non-hatching eggs and biparental males; the biparental males being produced by close-bred females which produce a much higher percentage of non-hatching eggs than either the unmated females or females mated with unrelated males. The increase in percentage of non-hatching eggs from close-bred females and the decrease in percentage of biparental offspring indicates that in such females there is a diminution in responsiveness to oviposition stimuli and a corresponding increase in the absorption of the ripe ovarian eggs.

In ectoparasitic species, oosorption probably proceeds with greater rapidity than oogenesis (Flanders, 1942). Slowness in response to oviposition stimuli may result in the deposition of slightly absorbed eggs of low viability. A decrease in oviposition rate may account for the fact noted by Whiting (1940) that the percentage of nonhatching eggs deposited by *M. hebetor* increases with the age of the ovipositing female.

Whiting (1940) points out that in *M. hebetor* embryonic development occurs in almost every nonhatching egg. Consequently, it seems probable that eggs that have not regressed beyond a certain point may hatch and, if able to feed on their host, complete their development.

There exists, therefore, the possibility that individuals from slightly regressed fertilized eggs may have undergone sex reversal prior to feeding on the host as a result of undernourishment during embryonic development.

In the ectoparasite *Melittobia chalybii,* in which close breeding is normal, a high percentage of nonhatching eggs is often observed. In unmated females, however, the percentage is much greater since, unlike *Microbracon hebetor,* mating is a prerequisite of normal oviposition. Females mated with males of a different species also oviposit normally. Since unmated females, females mated to males of another species and normally mated females may produce equal numbers of male offspring, it seems evident that in *M. chalybii* biparental males rarely, if ever, occur (Schmieder, 1938). In this species the relation between oviposition and oosorption may be such that regressed eggs capable of development are not deposited.

It is possible that the production of diploid males in the Hymenoptera is a specific phenomenon, correlated with rate of oosorption in the ovary.

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